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Pharmacokinetic and pharmacodynamic assessment of valganciclovir in solid organ transplant recipients

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de l'Université de Lausanne par

Nancy Perrottet

Pharmacienne diplômée de la Confédération helvétique
de l'Université de Lausanne

Jury

Prof. Alfio Marazzi, Président
Dr Thierry Buclin, PD, MER, Directeur de thèse
Prof. Jérôme Biollaz, co-Directeur de thèse
Prof. Pascal Meylan, Expert
Dr Nicolas Schaad, PD, Expert

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Directeur de thèse	Monsieur	Dr Thierry	**Buclin**
Co-directeur de thèse	Monsieur	Prof. Jérôme	**Biollaz**
Experts	Monsieur	Prof. Pascal	**Meylan**
	Monsieur	Dr Nicolas	**Schaad**

le Conseil de Faculté autorise l'impression de la thèse de

Madame Nancy Perrottet

Pharmacienne diplômée de la Confédération Helvétique

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Pharmacokinetic and pharmacodynamic assessment of valganciclovir in solid organ transplant recipients

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pour Le Doyen
de la Faculté de Biologie et de Médecine

A. Marazzi

Prof. Alfio Marazzi

A ma famille

Aux personnes en attente d'un don d'organe

« La science cherche le mouvement perpétuel. Elle l'a trouvé : c'est elle-même. »

Victor Hugo

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Abstract

Pharmacokinetic and pharmacodynamic assessment of valganciclovir in solid organ transplant recipients

Valganciclovir (Valcyte®) is an orally administered ester prodrug of the standard anti-cytomegalovirus (CMV) drug ganciclovir. This drug enabled an important reduction of the burden of CMV morbidity and mortality in solid organ transplant recipients. Prevention of CMV infection and treatment of CMV disease requires drug administration during many weeks. Oral drug administration is therefore convenient. Valganciclovir has been developed to overcome the poor oral availability of ganciclovir, which limits its concentration exposure after oral administration and thus its efficacy. This prodrug crosses efficiently the intestinal barrier, is then hydrolyzed into ganciclovir, providing exposure similar to intravenous ganciclovir. Valganciclovir is now preferred for the prophylaxis and treatment of CMV infection in solid organ transplant recipients. Nevertheless, adequate dosage adjustment is necessary to optimize its use, avoiding either insufficient or exaggerate exposure related to differences in its pharmacokinetic profile between patients.

The main goal of this thesis was to better describe the pharmacokinetic and pharmacodynamic profile of valganciclovir in solid organ transplant recipients, to assess their reproducibility and their predictability, and thus to evaluate the current recommendations for valganciclovir dosage adjustment and the potential contribution of routine therapeutic drug monitoring (TDM) to patients' management. A total of 437 ganciclovir plasma concentration data from 65 transplant patients (41 kidney, 12 lung, 10 heart and 2 liver recipients, 58 under oral valganciclovir prophylaxis, 8 under oral valganciclovir treatment and 2 under intravenous ganciclovir) were measured using a validated chromatographic method (HPLC) developed for this study. The results were analyzed by non-linear mixed effect modeling (NONMEM).

A two-compartment model with first-order absorption appropriately described the data. Systemic clearance was markedly influenced by GFR, with further differences between graft types and sex (CL/GFR = 1.7 in kidney, 0.9 in heart and 1.2 in lung and liver recipients) with interpatient variability (CV%) of 26% and interoccasion variability of 12%. Body weight and sex influenced central volume of distribution (V_1 = 0.34 l/kg in males and 0.27 l/kg in females) with an interpatient variability of 20%. Residual intrapatient variability was 21%. No significant drug interaction influenced GCV disposition. VGC prophylactic efficacy and tolerability were good, without detectable dependence on GCV profile.

In conclusion, this analysis highlights the importance of thorough adjustment of VGC dosage to renal function and body weight. Considering the good predictability and reproducibility of GCV profile after oral VGC in solid organ transplant recipients, routine TDM does not appear to be clinically indicated. However, GCV plasma measurement may still be helpful in specific clinical situations such as documentation of appropriate exposure in patients with potentially compromised absorption, or lack of response to CMV disease treatment, or under renal replacement therapy.

Etude pharmacocinétique et pharmacodynamique du valganciclovir chez les transplantés d'organe

Le valganciclovir (Valcyte®) est un promédicament oral du ganciclovir qui est un anti-infectieux de référence contre les infections à cytomegalovirus (CMV). Cet antiviral a permis de réduire les effets délétères de cette infection jusqu'ici responsable d'une importante morbidité et mortalité chez les transplantés d'organe. La prévention et le traitement de l'infection à CMV sont donc nécessaires mais requièrent l'administration d'un agent antiviral sur une longue période. Un médicament administré par voie orale représente donc un avantage évident. Le valganciclovir a été développé dans le but d'améliorer la faible absorption orale du ganciclovir, et donc son efficacité. Cet ester valylique du ganciclovir traverse plus facilement la barrière gastro-intestinale, puis est hydrolysé en ganciclovir dans la circulation sanguine, produisant une exposition comparable à celle d'une perfusion intraveineuse de ganciclovir. De ce fait, le valganciclovir est devenu largement utilisé pour la prophylaxie mais aussi le traitement de l'infection à CMV. Néanmoins une utilisation optimale de ce nouveau médicament nécessite de bonnes connaissances sur son profil pharmacocinétique afin d'établir un schéma de dose adapté pour éviter tant une surexposition qu'une sous-exposition résultant des différences d'élimination entre les patients.

Le but de cette thèse a été d'étudier le profil pharmacocinétique et pharmacodynamique du valganciclovir chez les transplantés d'organe ainsi que sa reproductibilité et sa prédictibilité. Il s'agissait d'apprécier de manière critique le schéma actuellement recommandé pour l'adaptation des doses de valganciclovir, mais aussi la contribution éventuelle d'un suivi des concentrations sanguines en routine. Un total de 437 taux sanguins de ganciclovir ont été mesurés, provenant de 65 patients transplantés d'organe (41 rénaux, 12 pulmonaires, 10 cardiaques et 2 hépatiques, 58 sous une prophylaxie orale de valganciclovir, 8 sous un traitement de valganciclovir et 2 sous un traitement intraveineux). Une méthode de chromatographie liquide à haute performance a été développée et validée pour cette étude. Les résultats ont été ensuite analysés par modélisation non linéaire à effets mixtes (NONMEM).

Un modèle à deux compartiments avec absorption de premier ordre a permis de décrire les données. La clairance systémique était principalement influencée par le débit de filtration glomérulaire (GFR), avec une différence entre les types de greffe et les sexes (CL/GFR = 1.7 chez les greffés rénaux, 0.9 pour les greffés cardiaques et 1.2 pour le groupe des greffés pulmonaires et hépatiques) avec un variabilité inter-individuelle de 26% (CV%) et une variabilité inter-occasion de 12%. Le poids corporel ainsi que le sexe avaient une influence sur le volume central de distribution (V_1 = 0.34 l/kg chez les hommes et 0.27 l/kg chez les femmes) avec une variabilité inter-individuelle de 20%. La variabilité intra-individuelle résiduelle était de 21%. Aucune interaction médicamenteuse n'a montré d'influence sur le profil du ganciclovir. La prophylaxie avec le valganciclovir s'est révélée efficace et bien tolérée.

En conclusion, cette analyse souligne l'importance d'une adaptation de la dose du valganciclovir à la fonction rénale et au poids du patient. Au vu de la bonne reproductibilité et prédictibilité du profil pharmacocinétique du ganciclovir chez les patients transplantés recevant du valganciclovir, un suivi des concentrations sanguines en routine ne semble pas cliniquement indiqué. Néanmoins, la mesure des taux plasmatiques de ganciclovir peut être utile dans certaines situations particulières, comme la vérification d'une exposition appropriée chez des patients susceptibles d'absorption insuffisante, ou ne répondant pas au traitement d'une infection à CMV ou encore sous épuration extra-rénale.

Une étude de la pharmacocinétique et pharmacodynamie du valganciclovir chez les patients transplantés d'organe solide

Le valganciclovir est un précurseur capable de libérer du ganciclovir, récemment développé pour améliorer la faible absorption orale de ce dernier. Une fois le valganciclovir absorbé, le ganciclovir libéré dans la circulation sanguine devient efficace contre les infections à cytomégalovirus. Ce virus largement répandu est responsable de maladies insidieuses et parfois graves chez les personnes présentant une baisse des défenses immunitaires, comme les greffés d'organe recevant un traitement anti-rejet. Le ganciclovir est administré pendant plusieurs mois consécutifs soit pour prévenir une infection après la transplantation, soit pour traiter une infection déclarée. La facilité d'administration du valganciclovir par voie orale représente un avantage sur une administration du ganciclovir par perfusion, qui nécessite une hospitalisation. Toutefois, la voie orale peut être une source supplémentaire de variabilité chez les patients, avec un impact potentiel sur l'efficacité ou la toxicité du médicament. Le but de cette étude a été :

- de décrire le devenir de ce médicament dans le corps humain (dont l'étude relève de la discipline de la pharmacocinétique)
- de définir les facteurs cliniques pouvant expliquer les différences de concentration sanguine observées entre les patients sous une posologie donnée
- d'explorer les relations entre les concentrations du médicament dans le sang et son efficacité ou la survenue d'effets indésirables (dont l'étude relève de la discipline de la pharmacodynamie).

Cette étude a nécessité le développement et la validation d'une méthode d'analyse pour mesurer la concentration sanguine du ganciclovir, puis son application à 437 échantillons provenant de 65 patients transplantés d'organe solide (41 rénaux, 12 pulmonaires, 10 cardiaques et 2 hépatiques) recevant du valganciclovir. Les résultats des mesures effectuées ont été analysés à l'aide d'un outil mathématique afin d'élaborer un modèle du devenir du médicament dans le sang chez chaque patient et à chaque occasion.

Cette étude a permis d'évaluer chez des patients recevant le valganciclovir, la vitesse à laquelle l'organisme absorbe, distribue, puis élimine le médicament. La vitesse d'élimination dépendait étroitement de la fonction rénale, du type de greffe et du sexe alors que la distribution dépendait du poids et du sexe du patient. La variabilité non expliquée par ces facteurs cliniques était modérée et vraisemblablement sans conséquence clinique évidente soit sur l'efficacité ou la tolérance, qui se révèlent très satisfaisantes chez les patients de l'étude. Les observations n'ont pas révélé de relation entre les concentrations de médicament et l'efficacité thérapeutique ou la survenue d'effets indésirables, confirmant que les doses relativement faibles utilisées dans notre collectif de patients suffisaient à produire une exposition reproductible à des concentrations adéquates.

En conclusion, le profil (et par conséquent l'absorption) du valganciclovir chez les patients transplantés semble bien prédictible après une adaptation de la dose à la fonction rénale et au poids du patient. Un contrôle systématique des concentrations sanguines n'est probablement pas indiqué en routine, mais cette mesure peut présenter un intérêt dans certaines conditions particulières.

Publications and scientific communications

Some parts of the present work have been published in international journals or presented in Swiss and international congresses as oral or poster presentations.

Publications

N. Perrottet, A. Beguin, P. Meylan, M. Pascual, O. Manuel, T. Buclin, J. Biollaz, L.A. Decosterd.

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Journal of Chromatography B 2007; 852(1-2): 420-9.

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Ganciclovir exposure under a 450 mg daily dosage of valganciclovir for cytomegalovirus prevention in kidney transplantation: a prospective study.

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Poster presentations

P. Meylan, N. Perrottet, O. Manuel, L.A. Decosterd, T. Buclin, J.P. Venetz, M. Pascual. Pharmacokinetic and pharmacodynamic assessement of valganciclovir in the prophylaxis and treatment of CMV disease in solid organ transplant patients. *World Transplant Congress*, Boston (USA), July 22-27 2006.

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N. Perrottet, C. Robatel, P. Meylan, M. Pascual, M.M. Berger, L.A. Decosterd, T. Buclin. Disposition of valganciclovir during continuous renal replacement therapy in two lung transplant recipients. *American Transplant Congress*, Toronto (Canada), June 31-July 4 2008.

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Abbreviations

AcOH	Acetic acid
ACV	Aciclovir
AIDS	Acquired immune deficiency syndrome
ANOVA	Analysis of variance (used in statistics)
AST	American Society of Transplantation
AUC	Area under the curve (used in pharmacokinetic modeling)
AZA	Azathioprine
BLAST	Basic Local Alignment Search Tool
BMT	Bone marrow transplant recipient
BW	Bodyweight
C_A	Prefilter concentration
Card	Cardiopathy
CHUV	Centre Hospitalier Universitaire Vaudois (i.e. Lausanne University hospital)
CL	Clearance
CL_{CRRT}	Haemofiltration clearance
CL_F	Filtration clearance
CL/F	Apparent oral clearance
C_{max}	Maximum concentration
C_{min}	Minimum concentration
CMMG	9-carboxymethoxymethylguanine
CMV	Cytomegalovirus
CNS	Central nervous system
COTM	Cotrimoxazole
CRRT	Continuous renal replacement therapy
Cr_s	Serum creatinin
CSF	Cerebrospinal fluid
CST	Canadian Society of Transplantation
C_{trough}	Trough concentration
C_V	Postfilter concentration
CV	Coefficient of variation
CVVHF	Continuous veno-venous haemofiltration
D	Donor
dGTP	Deoxyguanosine triphosphate
EBV	Epstein-Barr virus
EDTA	Ethylene-diamine-tetra-acetic acid
EIA	Enzyme immunoassay
ELFA	Enzyme-linked fluorescent assay
ELISPOT	Enzyme-linked immunospot
F	Bioavailability
FDA	Food and Drug Administration (in the United States)
GCV	Ganciclovir
GFR	Glomerular filtration rate
GFR_{C-G}	Estimated glomerular filtration rate using Cockroft-Gault equation
GFR_{MDRD}	Estimated glomerular filtration rate using four-variable modification of diet in renal disease formula

HGT	Height
HIV	Human immunodeficiency virus
H_2O	Water (ultrapure)
HP	Hewlett-Packard® (now Agilent Technologies®)
HPLC	High performance liquid chromatography
HS	Healthy subject
HT	Heart transplant recipient
HUG	Hôpitaux Universitaires de Genève (i.e. Geneva University hospitals)
IC_{50}	Inhibitory concentration 50%
IC95%	Confidence interval 95%
ICAL	Calcineurin inhibitors
ID	Internal diameter (for chromatography columns)
IFN-γ	Interferon-gamma
Ig	Immunoglobulin
I-GVHD	Intestinal graft-versus-host disease
IL	Interleukin
IOV	Interoccasion variability
IS	Internal standard (in analytical chemistry methods)
iv	Intravenous (for drug administration)
k_a	Absorption rate constant
K-PT	Kidney-pancreas transplant recipient
KT	Kidney transplant recipient
LC	Liquid chromatography
LiT	Liver transplant recipient
LLOQ	Lower limit of quantification
LOD	Limit of detection
LOQ	Limit of quantification
LuT	Lung transplant recipient
λ_1	Initial rate constant
λ_z	Terminal rate constant
MeCN	Acetonitrile
MIC	Minimum inhibitory concentration
MMF	Mycophenolate mofetil
MPS	Mycophenolic sodium
NaCl	Sodium chloride
NM-TRAN	NONMEM translator
NONMEM	NONlinear Mixed Effects Model
OAT	Organic anion transporter
OATI	Organic anion transporter inhibitor
OF	Objective function (in NONMEM, corresponding to -2 log likelihood)
PCL	Division of Clinical Pharmacology & Toxicology (at CHUV)
PCR	Polymerase chain reaction
PD	Pharmacodynamics
PEPT	Intestinal peptide transporter
P-GCV	Ganciclovir monophosphate
PK	Pharmacokinetics
PP-GCV	Ganciclovir biphosphate

PPP-GCV	Ganciclovir triphosphate
PTLD	Post-transplant lymphoproliferative disease
Q	Intercompartmental clearance
QC	Quality control
qd	Daily
R	Recipient, Recovery (in analytical part)
RBC	Red blood cell
RI	Patients with renal insufficiency
RT	Room temperature
S_C	Sieving coefficient
SCT	Stem cell transplant recipient
SD	Standard deviation
SE	Standard error
SOT	Solid organ transplant recipient
$t_{1/2}$	Half-life of elimination
$t_{1/2a}$	Half-life of absorption
TCA	Trichloroacetic acid
TDM	Therapeutic drug monitoring
t_{max}	Time corresponding to concentration maximum
UV	Ultraviolet
V_1	Central volume of distribution
V_2	Peripheral volume of distribution
VCA BAIA	Viral Capsid Antigen bead array immunoassay
VCA IF	Viral Capsid Antigen immunofluorescence
V_d	Volume of distribution
V/F	Apparent volume of distribution
VGC	Valganciclovir
V_{ss}	Volume of distribution at steady-state
V_z	Terminal volume of distribution

NONMEM Symbols

Δ	Delta: difference
ε	Epsilon: residual error of an observation *y*, also called random effect parameter
η	Eta: interindividual, random error in NONMEM models
ω	Omega: standard deviation of parameters θ generated by NONMEM
σ	Sigma: standard deviation of residual error ε
θ	Theta: fixed effects parameters generated by NONMEM
i	Observation
j	Subject (i.e. individual)
X	Covariates

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Chapter 1 : General introduction

Presentation of chapter 1

What is cytomegalovirus (CMV) infection in transplant recipients all about? What are the biological and pharmacological aspects underlying the medical use of agents such as ganciclovir and valganciclovir?

This chapter presents a general introduction, where the research subject is situated in its biological and medical background. Considerations on the risk of infection in organ transplant recipients are reviewed and set into patient management perspective.

Ganciclovir and its prodrug, valganciclovir, are presented with their historical development, mechanism of action, advantages and limitations. Ganciclovir is effective against CMV after intravenous administration, but its effect is limited after oral administration due to poor oral bioavailability. Valganciclovir was thus recently developed to overcome the limited absorption and convenience of ganciclovir. However, oral route is an additional source of variability compared to intravenous administration. This issue will be one of the main questions addressed by this work.

1. General Introduction

In complex medical conditions, multidisciplinary work plays a key role in patients' management, as the case of transplant recipients. After an organ transplantation, patients need an effective immunosuppressive treatment to avoid graft rejection. Immunosuppression favours various opportunistic infections (viral, bacterial, fungal and also parasitic), which thus require specific prophylaxis or treatment. The risk of infection is depending on the type and intensity of immunosuppression, the amount of exposure to potential pathogens and others factors contributing to patients' susceptibility. The management of solid organ transplant recipients thus represents a challenging equilibrium between effective immunosuppression and avoidance of infections.

1.1. Immunosuppressive therapy in solid organ transplant recipients

Immunosuppressive regimens are composed of induction and maintenance therapies. Monoclonal anti-IL-2Rα receptor antibodies or polyclonal anti-T-cell antibodies are used as a rapid and potent immunosuppression method to prevent the acute rejection reaction. Maintenance regimens consist usually of a triple immunosuppressive therapy, involving one drug acting on immunophilins (either calcineurin inhibitors such as tacrolimus and cyclosporine or sirolimus), one antimetabolic agent (mycophenolate or azathioprine) and corticosteroids. The maintenance of immunosuppressive therapy is achieved by combining agents with different but complementary mechanism of action to maximize overall effectiveness while minimizing morbidity and mortality associated with each class of agent. The adverse effects of these drugs include, among others, an increased risk of developing opportunistic infections.

1.2. Cytomegalovirus infection in solid organ transplant recipients

Cytomegalovirus (CMV) is one of the most common viral infection complicating solid organ transplantation. It is associated with both direct and indirect harmful effects. Direct effects (acute) include tissue injury and clinical symptoms, while indirect effects (acute or chronic) result from an increased risk of other opportunistic infections, of Epstein-Barr virus (EBV)

associated post-transplant lymphoproliferative disease (PTLD) and of allograft rejection and injury [1].

CMV belongs to the family of herpesviruses (besides herpes simplex viruses 1 and 2 and varicella zoster virus). It is a double-stranded DNA virus (figure 1) which has the ability to remain latent within the body over long periods. CMV primary infection in healthy individuals will only produce few symptoms (such as fever, sore throat, fatigue) and recurrent disease rarely occurs outside immune system depression, such as encountered in transplant recipients under immunosuppressive treatment or in patients with advanced AIDS. Infectious CMV particles may be shed intermittently in the body fluids (urine, saliva, blood, tears, semen, and breast milk) by any previously infected person without any detectable signs, and without causing symptoms. Thus, transmission occurs from person to person through contact with body fluids, but also through graft transplantation.

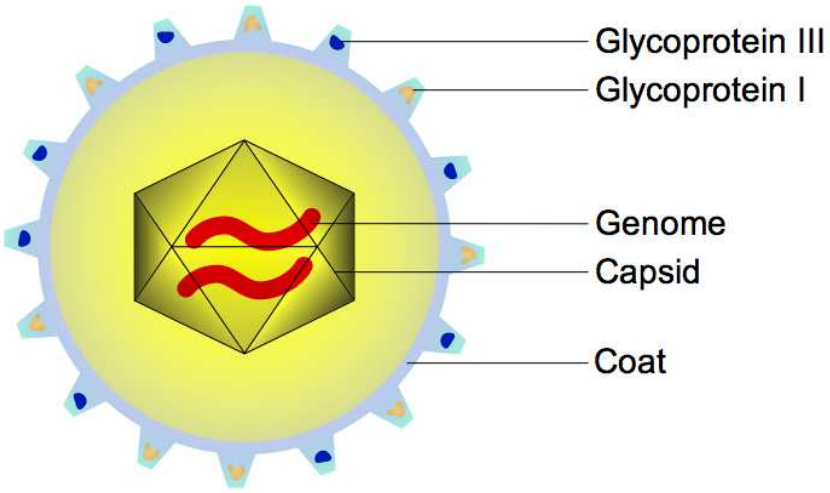

Figure 1: Scheme of cytomegalovirus [Emmanuel Boutet]

The ability to control CMV infection is mediated by virus specific CD4 and CD8 T-cells [2]. CMV reactivation from latency in cellular reservoirs (such as leukocytes, epithelial cells of salivary glands and cervix) can be induced by various factors such as antilymphocyte antibodies used for the induction immunosuppressive therapy, as well as systemic infection and inflammation, and also graft rejection itself. Spread of the virus is favoured by immunosuppressive treatments such as tacrolimus, cyclosporine, and corticosteroids, which depress the host's antiviral immune response [3]. Therefore, solid organ transplant recipients with latent CMV (R+) as well as CMV-naive recipients whose organ donor is CMV-experienced (D+/R-) are at risk of developing a CMV infection. In the latter case, the risks and potential seriousness are higher, as the recipient will experience a primary infection episode in the absence of pre-existing immunity.

A CMV infection can either be asymptomatic or lead to a CMV syndrome or tissue-invasive CMV disease (colitis, hepatitis, pneumonia, central nervous system disease, etc). As the onset of CMV infection usually takes place during the first months after transplantation [1], two strategies have been developed to prevent CMV disease during this period: *CMV infection prophylaxis*, which consists in the administration of antiviral agents to all recipients considered at risk for CMV disease, or *CMV infection pre-emptive therapy*, in which antivirals are administered only in response to an early laboratory (significant viremia load) or clinical trigger suggesting CMV replication. There is actually no consensus regarding the best method to prevent harmful CMV infection [4]. Indeed to date, no randomized control trials have compared both strategies. Nevertheless, two evidence-base guidelines for the management of CMV in solid organ transplantation have been published by the American Society of Transplantation (AST) and the Canadian Society of Transplantation (CST) [5,6]. They recommend universal prophylaxis for solid organ transplant recipients at high risk for CMV disease (donor is CMV-experienced and the recipient is naïve for CMV, i.e. D+/R- and lung, heart-lung R+) and either universal prophylaxis or pre-emptive therapy for those at a moderate risk (R+ kidney, liver, pancreas, heart recipients) or at low risk (D-/R+ not receiving induction therapy). AST also advises that graft function, state of immunosuppression, transplant centre and patients resources are considered while choosing between universal prophylaxis and pre-emptive therapy [5]. In the transplantation centre of Lausanne, universal prophylaxis is used in every kidney, lung and heart recipient known for CMV experience (R+) or whose donor is CMV-experienced (D+); and only in (D+/R-) liver recipients.

1.3. Ganciclovir & valganciclovir

Ganciclovir is the gold standard for the treatment of CMV infection. This nucleoside analogue of endogenous deoxyguanosine triphosphate (dGTP) is effective in vitro against herpesviruses but is mainly used against CMV infection. Ganciclovir, activated by phosphorylation into ganciclovir triphosphate, competes with dGTP as a substrate for viral DNA polymerase. Incorporation of ganciclovir triphosphate and reduction of dGTP inclusion into growing chains of viral DNA slows down their extension, thereby inhibiting viral replication. The selectivity of ganciclovir for CMV infected cells is attributed to a weaker inhibition by ganciclovir triphosphate of cellular DNA polymerase compared to viral DNA polymerase, and by the rather specific accumulation of ganciclovir triphosphate in CMV-infected cells [7]. In fact, ganciclovir triphosphate is produced rather specifically in infected cells because ganciclovir has a high affinity for a CMV viral protein kinase, which catalyzes its phosphorylation into a monophosphate derivative, while further phosphorylations are catalyzed by cellular kinases.

Intravenous ganciclovir was approved by the Food and Drug Administration (FDA) as an orphan drug in 1989. This drug was initially developed to reduce the burden of CMV infection in patients with AIDS, but was rapidly used in transplant recipients as well. Its mandatory administration through the intravenous route was nevertheless inconvenient for maintenance treatment in CMV retinitis. Fifteen years later, oral ganciclovir was commercialized. This new form of ganciclovir was used for maintenance treatment of CMV retinitis in patients with AIDS and for the prophylaxis of CMV infection in solid organ transplant recipients. However, the pharmacokinetic profile of oral ganciclovir was not optimal due to its poor oral availability (<10%). Despite the administration of high daily doses (1g three times per day), ganciclovir exposure was suboptimal and highly variable leading to CMV prophylaxis failure or emergence of resistant virus strains [8]. Thus the development of a better absorbed derivative was necessary. In 2001, an oral prodrug of ganciclovir, named valganciclovir, became available. This valine ester of ganciclovir was characterized by a much higher oral availability (about 10 times) compared to oral ganciclovir. One single dose per day produced a higher blood concentration exposure than obtained after three large dose of oral ganciclovir, and even a similar exposure compared to intravenous ganciclovir. Thus oral valganciclovir could offer significant advantages over both oral and intravenous ganciclovir. In fact, it should improve exposure and simplify dose administration schedule, thus ameliorating patients' adherence. It could reduce the length of hospitalization in patients treated for a CMV infection or disease. Due to those advantages, valganciclovir is now preferred in the transplantation centre of Lausanne for the prophylaxis of CMV infection in solid organ transplant patients, and is also used as an alternative to iv ganciclovir in the treatment of CMV infection.

Whereas valganciclovir has been developed for its good oral absorption, one should wonder whether its absorption is reproducible both in a given patient and between different patients. As with oral ganciclovir, if valganciclovir absorption is reduced for any reason, ganciclovir exposure can be insufficient to avoid or treat CMV infection, which would favour the emergence of resistance. On the other hand, ganciclovir is extensively eliminated by the kidney and intravenous ganciclovir dosage is known to require adjustment with respect to the degree of renal insufficiency. Overexposure to ganciclovir after oral valganciclovir in patients with reduced elimination due to renal failure could induce adverse effects. The manufacturer thus gives similar recommendations for dose adjustment in renal dysfunction. Furthermore, this drug is expensive and is administered during weeks to months. For all those reasons, correct dose adjustment is highly desirable for optimizing the use of this oral produg of ganciclovir, avoiding both insufficient and exaggerated exposure.

Therapeutic drug monitoring i.e. the feed-back adjustment of drug dosage based on measurements of circulating concentrations, is a useful tool to check drug exposure levels, and to individualize the treatment. Solid organ transplant recipients already benefit from this approach for the individualization of tacrolimus or cyclosporine dosages. Valganciclovir could also represent a good candidate for such a monitoring, as this drug is in the early phase of use in new indications.

1.4. Objectives of the thesis

The main goal of this thesis was to better describe the pharmacokinetic and pharmacodynamic profiles of valganciclovir in solid organ transplant recipients, in order to assess their reproducibility and their predictability and thus evaluate the adequacy of actual valganciclovir dosage adjustment and the potential contribution of routine therapeutic drug monitoring in patients' management. Such knowledge is expected to contribute to optimize valganciclovir utilization for the prophylaxis or the treatment of CMV infection in solid organ transplant recipients in the Organ Transplantation centre of Lausanne.

To that endeavour, the concrete objectives of this work were:

- to review current knowledge accumulated in the medical literature on the pharmacokinetic and pharmacodynamic characteristics of valganciclovir (chapter 2)
- to develop and validate an analytical method for ganciclovir plasma level quantification (chapter 3)
- to perform a population pharmacokinetic study in solid organ transplant recipients, thus enabling an evaluation of the variability in ganciclovir exposure and of the suitability of a therapeutic drug monitoring approach (chapter 4) *[controlled-trials.com, ISRCTN Register, number ISRCTN06404801]*
- to describe valganciclovir profile in specific clinical situations (e.g. conditions possibly compromising absorption or elimination) (chapter 5)

1.5. References

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Chapter 2 : Literature review

Presentation of chapter 2

What is currently known on the pharmacokinetics (PK) and pharmacodynamics (PD) of valganciclovir? To what extent can the results gathered for ganciclovir help us to understand and predict the characteristics of valganciclovir?

This chapter is a review article focusing on clinical PK and PD characteristics of valganciclovir and ganciclovir. PK studies on both agents are systematically reviewed and the results summarized using the general methods of meta-analysis. Variability and patients' characteristics influencing PK profile are appraised, along with concentration-efficacy and -toxicity relationships. The potential role of therapeutic drug monitoring of valganciclovir in the management of transplant recipients is evaluated based on this review.

This literature review will also help us to discuss the results of our clinical study on the PK and PD of valganciclovir (chapter 4) and also to interpret ganciclovir plasma concentrations measured during the study (chapter 3).

Valganciclovir in solid organ transplant patients: pharmacokinetic and pharmacodynamic characteristics and clinical interpretation of plasma concentration measurements

N. Perrottet[1], L.A. Decosterd[1], P. Meylan[2], M. Pascual[3], J. Biollaz[1], T. Buclin[1]

[1] Division of Clinical Pharmacology and Toxicology, University Hospital, Lausanne, Switzerland

[2] Microbiology Institute, University Hospital, Lausanne, Switzerland

[3] Organ Transplantation Center, University Hospital, Lausanne, Switzerland

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2. Pharmacokinetic and pharmacodynamic characteristics and clinical interpretation of plasma concentration measurements

2.1. Abstract

Valganciclovir and ganciclovir are widely used for the prevention of cytomegalovirus (CMV) infection in solid organ transplant recipients, with a major impact on patients' morbidity and mortality. Oral valganciclovir, the ester prodrug of ganciclovir, has been developed to enhance the oral bioavailability of ganciclovir. It crosses the gastrointestinal barrier through peptide transporters and is then hydrolysed into ganciclovir. This review aims to describe the current knowledge of the pharmacokinetic and pharmacodynamic characteristics of this agent, and to address the issue of therapeutic drug monitoring.

Based on currently available literature, ganciclovir pharmacokinetics in adult solid organ transplant recipients receiving oral valganciclovir are characterized by bioavailability of 66 ± 10% (mean ± SD), a maximum plasma concentration of 3.1 ± 0.8 mg/l after a dose of 450 mg and of 6.6 ± 1.9 mg/l after a dose of 900 mg, a time to reach the maximum plasma concentration of 3.0 ± 1.0 h, area under the plasma concentration-time curve values of 29.1 ± 5.3 mg·h/l and 51.9 ± 18.3 mg·h/l (after 450 mg and 900 mg, respectively), apparent clearance of 12.4 ± 3.8 l/h, an elimination half-life of 5.3 ± 1.5 h and an apparent terminal volume of distribution of 101 ± 36 l. The apparent clearance is highly correlated with renal function, hence the dosage needs to be adjusted in proportion to the glomerular filtration rate. Unexplained interpatient variability is limited (18% in apparent clearance and 28% in the apparent central volume of distribution). There is no indication of erratic or limited absorption in given subgroups of patients; however, this may be of concern in patients with severe malabsorption.

The *in vitro* pharmacodynamics of ganciclovir reveal a mean concentration producing 50% inhibition (IC_{50}) among CMV clinical strains of 0.7 mg/l (range 0.2–1.9 mg/l). Systemic exposure of ganciclovir appears to be moderately correlated with clinical antiviral activity and haematotoxicity during CMV prophylaxis in high-risk transplant recipients. Low ganciclovir plasma concentrations have been associated with treatment failure and high concentrations with haematotoxicity and neurotoxicity, but no formal therapeutic or toxic ranges have been validated.

The pharmacokinetic parameters of ganciclovir after valganciclovir administration (bioavailability, apparent clearance and volume of distribution) are fairly predictable in adult transplant patients, with little interpatient variability beyond the effect of renal function and bodyweight. Thus ganciclovir exposure can probably be controlled with sufficient accuracy by thorough valganciclovir dosage adjustment according to patient characteristics. In addition, the therapeutic margin of ganciclovir is loosely defined. The usefulness of systematic therapeutic drug monitoring in adult transplant patients therefore appears questionable; however, studies are still needed to extend knowledge to particular subgroups of patients or dosage regimens.

2.2. Introduction

Cytomegalovirus (CMV) used to rank as the primary cause of morbidity and mortality among solid organ transplant recipients [1] and is associated with both direct and indirect clinical manifestations. Without antiviral coverage, the incidence of CMV disease at 2 months post-transplant reaches up to 45% among high-risk patients (donor CMV seropositive/recipient CMV seronegative, D+/R-) [2]. Thus either universal prophylaxis or pre-emptive therapy with ganciclovir or its prodrug valganciclovir are recommended to prevent CMV infection [3]. Prophylaxis with antiviral agents significantly reduces the risks of CMV infection, CMV disease and all-cause mortality, as confirmed in a recent meta-analysis [4]. Indeed, the incidence of CMV infection in untreated patients, ranging from 27% to 100%, decreased by 39% with antiviral prophylaxis. Similarly, the incidence of CMV disease, ranging from 11% to 90%, decreased by 58%. In terms of the number-needed-to-treat, to prevent one case of CMV infection requires that 2–7 patients receive the treatment [4]. A 3-month course of valganciclovir prophylaxis markedly reduces the probability of CMV breakthrough viraemia among D+/R- patients to below 3% during the treatment; however, late-onset CMV diseases still occur in up to 17% over the first year post-transplant [5].

Valganciclovir, the prodrug L-valine ester of ganciclovir, has been developed to overcome the poor oral bioavailability of ganciclovir, which limits its exposure after oral administration. The high bioavailability of ganciclovir after valganciclovir administration is related to the recognition of the prodrug as a substrate by the intestinal peptide transporter PEPT1, whereas ganciclovir itself is not recognized [6]. Valganciclovir is readily hydrolysed by an amino-acid ester hydrolase (named valacyclovirase) to ganciclovir and released into the bloodstream (figure 1) [7,8].

Ganciclovir pharmacokinetics have been well characterized in different populations of adults, including healthy subjects, HIV-infected patients and transplant recipients. Ganciclovir

clearance is highly dependent on renal function as this drug is extensively eliminated by the kidney. Thus its dosage needs to be adjusted with respect to kidney function.

Ganciclovir pharmacodynamic properties are not different when delivered as valganciclovir or as intravenous ganciclovir, but what about the pharmacokinetic characteristics and variability of ganciclovir after administration of its prodrug? And what is their impact on therapeutic efficacy and safety? Routine therapeutic monitoring (TDM) of ganciclovir has been evaluated as unwarranted in solid organ transplant patients, considering its highly predictive disposition after intravenous infusion [9]; however, this question has not been addressed after valganciclovir administration. This review aims to describe the current knowledge of ganciclovir pharmacokinetics and pharmacodynamics after valganciclovir administration, to evaluate the potential contribution of TDM in the management of adult solid organ transplant recipients receiving valganciclovir prophylaxis or treatment, and to provide keys to the interpretation of ganciclovir plasma results measured in plasma from patients receiving oral valganciclovir therapy.

2.3. Pharmacokinetic characteristics of ganciclovir given as oral oral valganciclovir

The specific aspects of ganciclovir pharmacokinetics after valganciclovir administration essentially pertain to the oral absorption and biotransformation of the prodrug into ganciclovir. Beyond this step, ganciclovir is expected to follow similar disposition to that described after intravenous administration; therefore this review covers only aspects of ganciclovir pharmacokinetics relevant to understanding of the clinical pharmacology of valganciclovir. A literature search was performed on MEDLINE (from 1966 to February 2008 using the following key words: ‘valganciclovir’, ‘ganciclovir’, ‘pharmacokinetics’, ‘pharmacodynamics’, ‘therapeutic drug monitoring’, ‘toxicity’, ‘neutropenia’, ‘leucopenia’, ‘neurotoxicity’, and ‘hepatotoxicity’), combined with a search of references listed in relevant articles. Pharmacokinetic parameters were systematically reviewed, average values of each parameter were calculated by arithmetic means of the estimates reported in articles, and their precision was estimated by pooled variance calculation. Correlations between pharmacokinetic parameters and patient factors were systematically reviewed.

The pharmacokinetic properties of ganciclovir studied in adult patients after oral valganciclovir and intravenous ganciclovir are summarized in tables 1 and 2, respectively.

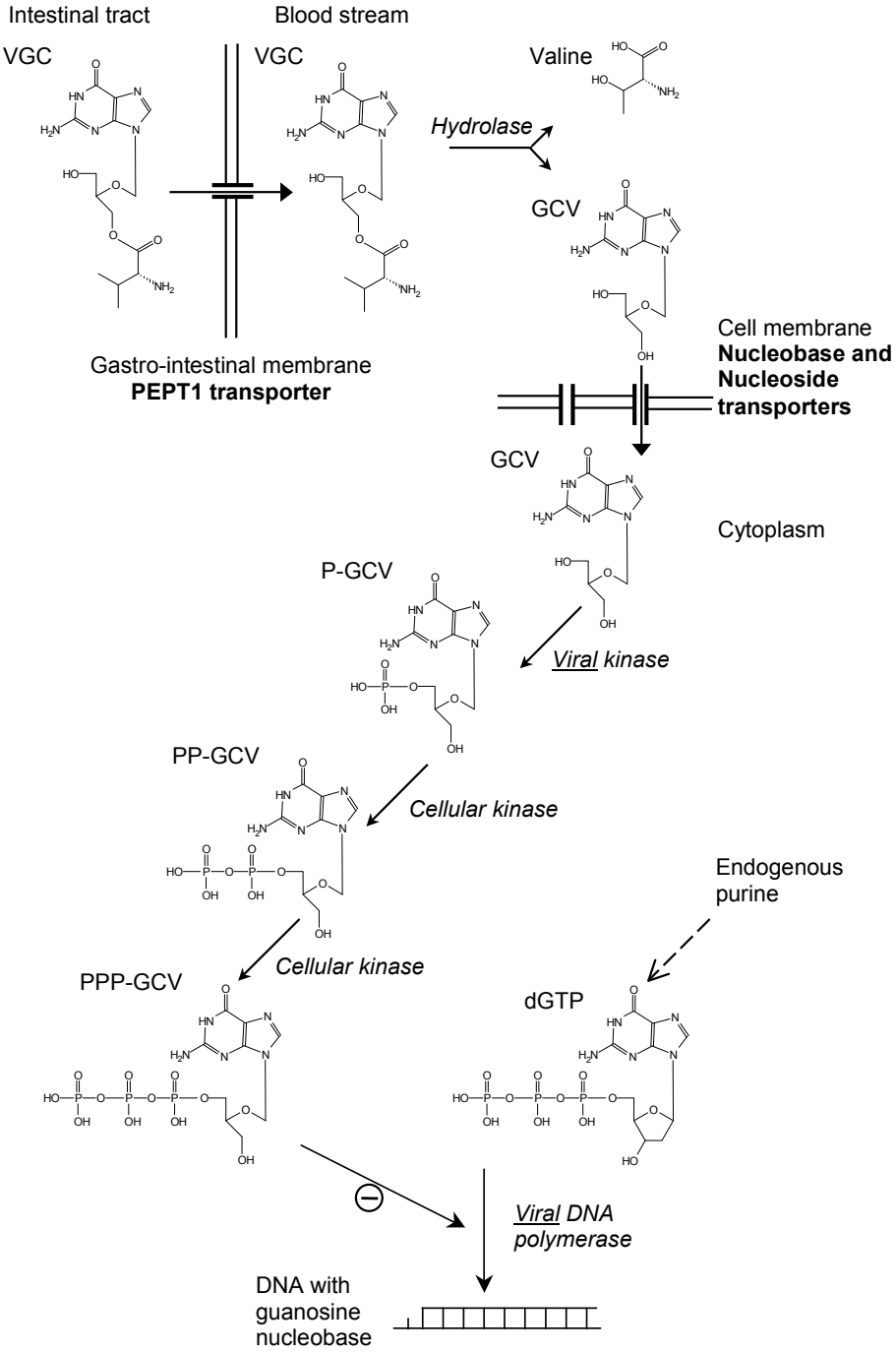

Figure 1: The valganciclovir pathway: form absorption to bioactivation. dGTP = deoxyguanosine triphosphate, GCV = ganciclovir, PEPT1 = intestinal peptide transporter, P-GCV = ganciclovir monophosphate, PP-GCV = ganciclovir biphosphate, PPP-GCV = ganciclovir triphosphate, VGC = valganciclovir.

2.3.1. Oral absorption and biotransformation of valganciclovir

The average value of the absolute oral bioavailability (F) of ganciclovir after oral administration of valganciclovir amounted to 66% ± 10% across seven trials, which determined this parameter in 138 patients. Oral availability was determined by expressing valganciclovir doses as the ganciclovir equivalent to account for valine subtraction (factor: x255.23/354.36). The consistency between studies was good, except for one study, which reported an oral availability of 88% [14]; however, this value was estimated by population pharmacokinetic analysis, whereas other studies used an individual approach. Little is known about the absorption kinetics of valganciclovir, which is known to briefly appear in the circulation at fairly low concentrations (maximum plasma concentration [C_{max}] 0.2 mg/l after a 900 mg dose) as it undergoes rapid transformation into ganciclovir [15]. Only two population pharmacokinetic studies reported absorption/metabolism rate constants, which were 0.36 h^{-1} [13] and 0.96 h^{-1} [14], with lag times of 0.66 h [13] and 0.39 h [14], respectively. The average C_{max} of ganciclovir amounted to 3.1 ± 0.8 mg/l after a dose of 450 mg and to 6.6 ± 1.9 mg/l after 900 mg. The time to reach the C_{max} (t_{max}) appeared to be reached sooner in healthy subjects or HIV/AIDS-infected patients (1.6 ± 0.6 h) than in transplant patients (3.0 ± 1.0 h). The mean values of the area under the plasma concentration-time curve (AUC) over a dosing interval (AUCτ) of ganciclovir reported in transplant recipients receiving 450 mg and 900 mg of valganciclovir were 2.5 times higher and 1.7 times higher, respectively, than the values reported in healthy subjects and HIV/AIDS-infected patients given the same dosage. This difference probably arises from a difference in clearance rather than in bioavailability (see section 2.1). Food intake before valganciclovir administration increases ganciclovir exposition (AUC from 0 to 24 h [AUC_{24}]) statistically by average values of 24% and 30% for doses of 450 mg and 900 mg, respectively. In the fed state, the AUC_{24} increased proportionally with the dose [18].

2.3.2. Ganciclovir disposition

After intravenous administration of ganciclovir 2.5 mg/kg and 5 mg/kg, the average C_{max} values reached 4.7 ± 1.2 mg/l and 10 ± 2.7 mg/l, respectively. AUC values reported after a dose of 5 mg/kg were 1.7 times higher in transplant patients than in healthy subjects or HIV/AIDS-infected patients. Ganciclovir clearance reached 8.0 ± 2.9 l/h in transplant patients (determined across ten trials totalling 147 patients) and 13.6 ± 4.2 l/h in healthy subjects and HIV/AIDS-infected patients (determined across nine trials totalling 147 subjects). Accordingly, the reported elimination half-life ($t_{½}$) values were longer in transplant patients than in healthy subjects and HIV/AIDS-infected patients (4.7 ± 1.4 h vs 3.7 ± 1.1 h). The

terminal volume of distribution was 50 ± 16 l in transplant recipients (determined across eight trials totalling 132 patients) and 71 ± 37 l in healthy subjects and HIV/AIDS-infected patients (determined across eight trials totalling 140 subjects).

Ganciclovir disposition was most often described using a two-compartment approach. The mean absolute central volume of distribution amounted to 17 ± 3 l in five kidney transplant recipients [24] and to 11 ± 7 l in five bone marrow transplant recipients [28]. Ganciclovir is minimally bound to plasma proteins (1–2%) over a concentration range of 0.5–51 mg/l [44]. Plasma ganciclovir quickly equilibrates with red blood cells, with an erythrocyte/plasma ratio of about 0.8 [45].

The apparent oral clearance of ganciclovir (CL/F) after valganciclovir administration is also about 50% lower in transplant recipients than in healthy subjects and HIV/AIDS-infected patients. The average CL/F in transplant recipients (determined across six trials totalling 266 patients) reached 12.4 ± 3.8 l/h, which was congruent with intravenous ganciclovir clearance after correction for oral bioavailability of 65% (8 l/h). The average $t_{½}$ in transplant recipients was 5.3 ± 1.5 h. The apparent terminal volume of distribution reached 141 ± 39 l in healthy subjects and HIV/AIDS-infected patients (determined across four trials totalling 110 patients) and 101 ± 36 l in transplant recipients (determined across five trials totalling 253 patients). These values were also congruent with an intravenous volume of distribution of ganciclovir (V_d) after correction for oral bioavailability of 65%. Ganciclovir disposition after oral valganciclovir administration is also consistent with a two-compartment model. The mean apparent central and peripheral volumes of distribution (V_1 and V_2) amounted to 25 ± 3 l and 49 ± 3 l, respectively, in 160 solid organ transplant recipients [13], similar to the values reported in ten transplant patients infected with CMV (V_1 = 34 ± 5 l and V_2 = 30 ± 9 l) [14]. The intercompartmental clearance values reported by those two studies were 12.0 ± 2.5 l/h [13] and 9.0 ± 2.0 l/h [14].

Figure 2 shows a typical concentration-time profile of ganciclovir after oral administration of valganciclovir 900 mg (based on pooled pharmacokinetic parameters).

2.3.3. Known influences

Patient population

Healthy subjects and HIV/AIDS-infected patients receiving oral valganciclovir display similar ganciclovir pharmacokinetic parameters [20], whereas transplant patients receiving oral valganciclovir or intravenous ganciclovir display greater exposure (AUC), $t_{½}$ and apparent or absolute terminal volumes of distribution and lesser apparent or absolute clearance.

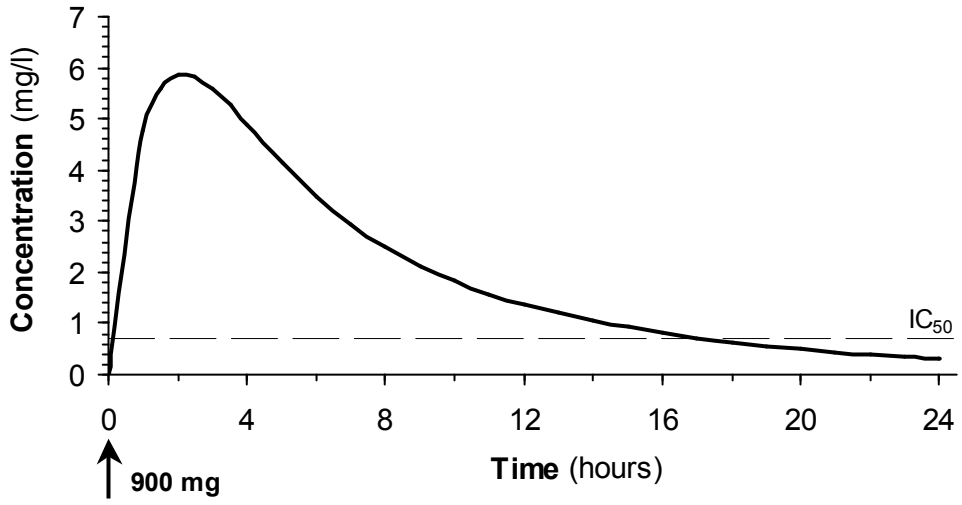

Figure 2: Typical pharmacokinetic profile of ganciclovir after oral administration of valganciclovir 900 mg in an 80 kg patient with a GFR of 80 ml/min. IC_{50} = concentration producing 50% inhibition of viral replication.

In the articles that were reviewed, the mean clearance in transplant patients was approximately half that in HIV/AIDS-infected patients. A population study reported an even smaller ratio despite correction for the glomerular filtration rate (GFR); however, the investigators recognized a possibly confounding analytical problem [46]. Transplant patients may thus require lower dosages than HIV-infected patients to reach the same level of drug exposure even beyond correction for the GFR. A subset of HIV-infected patients with CMV retinitis were shown to clear ganciclovir about 40% faster than HIV-infected patients only shedding CMV into urine [46,47], suggesting a potential impact of the inflammatory state or concurrent medications on drug elimination. Exposure to ganciclovir from oral valganciclovir was similar among liver, heart and kidney transplant recipients [13]. Allogenic stem cell transplant patients without and with intestinal graft-versus-host disease (I-GVHD grade I–II) who were receiving valganciclovir displayed similar oral availability, which appeared higher than that in healthy subjects, possibly related to intestinal tract damage following myeloablative conditioning therapy, which may increase absorption of the prodrug [16].

Comorbidities

Renal insufficiency has a determinant influence on ganciclovir pharmacokinetics, as kidney function is by far the most significant factor influencing ganciclovir clearance. This drug is extensively eliminated through both glomerular filtration and tubular secretion. An average of 85% (range 73–99%) of the dose is recovered in the urine after intravenous ganciclovir

administration, and no metabolism has been reported so far. Sommadossi et al. [42] first observed accumulation of ganciclovir in patients with mild renal insufficiency (GFR 41 ± 22 ml/min). The AUC was three times higher than in patients with normal renal function. Czock et al. [20] also reported accumulation of ganciclovir after valganciclovir administration. In comparison with patients with normal renal function, the AUC was increased by a factor of 2 in patients with mild renal failure and by 3.5 and 9 in patients with moderate and severe renal impairment, respectively (table 2). A significant influence of renal function has been confirmed in several studies including population analyses (table 3), involving both intravenous ganciclovir and oral valganciclovir. In the five available studies, the correlation between absolute ganciclovir clearance and creatinine clearance was described by a slope ranging from 1.3 to 2.4 with a positive or negative or null intercept value. Such values are in accordance with significant tubular secretion of ganciclovir. Estimates obtained with population approaches give roughly similar numbers [46,47]. After valganciclovir administration, the apparent clearance of ganciclovir estimated by the population approach is about 2.5–3.6 times the creatinine clearance. Thus the ganciclovir and valganciclovir dosage has to be adjusted to the degree of renal insufficiency. This review roughly supports the scheme recommended by the manufacturer in HIV/AIDS patients, but there are indications suggesting that transplant patients may require smaller doses at a given rate of glomerular filtration, possibly as a consequence of their comedication (see section below).

Stem cell transplant patients with I-GVHD can have severe diarrhoea and decreased absorption, depending on the grade of the disease. While patients with stable I-GVHD (grade II–III) displayed sufficient ganciclovir exposure (AUC 52 ± 21 mg·h/l [38]) after a dose of valganciclovir 900 mg, one patient with severe I-GVHD (grade III) with severe malabsorption had reduced exposure (AUC 14.8 mg·h/l [16]). Because valganciclovir absorption can be significantly reduced in patients with severe I-GVHD associated with significant malabsorption, the authors state that intravenous ganciclovir should be used instead.

Further demographic characteristics

Bodyweight has been shown to be correlated with absolute and apparent clearance [13,46,47] as well as with the central volume of distribution [14,46,47] in population pharmacokinetic studies (table 3). Absolute clearance estimated by two different population approaches in the same patient population was 0.17–0.24 (l/h/kg) [adjusted to a GFR of 100 ml/min]. Moreover, the absolute central volume of distribution also depends on bodyweight (0.44 l/kg, determined across two trials totalling 63 patients). This implies that heavy patients

have both greater absolute clearance and a larger central volume of distribution. Sex further influences the apparent central volume of distribution, with women having a reduced volume (by 40% in a population pharmacokinetic study) [13]. However, this model did not include bodyweight as a covariate, therefore sex was partly included in this factor (as women usually have less lean body mass than men). Other factors such as age and race were not evaluated beyond their impact on the GFR and bodyweight.
Very light patients may require less valganciclovir than heavy patients to reach the same ganciclovir exposure, even once the dosage is adjusted for renal function.

Concurrent medications

Coadministration of probenecid in HIV-infected patients receiving oral ganciclovir decreases renal clearance of ganciclovir by 19%, leading to an increase in the AUC and $t_{½}$ [48]. In fact, probenecid inhibits organic anion transporters involved in active renal tubular secretion of ganciclovir and thus compromises the tubular secretion component of its renal clearance.

Trimethoprim administered in association with oral ganciclovir in a multiple-dose study in HIV-infected patients caused a significant 13% decrease in renal clearance of ganciclovir [49].
When a single dose of mycophenolate mofetil was administered with a dose of intravenous ganciclovir to kidney transplant recipients, renal clearance of ganciclovir decreased slightly (12%); however, this difference did not reach statistical significance [22].
No statistically significant effects on renal clearance of ganciclovir were reported after oral administration associated with zidovudine, didanosine, zalcitabine or stavudine [48,50,51], nor with concomitant or alternating administration of foscarnet with oral ganciclovir for the treatment of AIDS-related CMV retinitis [36].
No significant effect of prophylactic intravenous ganciclovir on ciclosporin concentrations was detected in a retrospective study after heart transplantation [52]. No study has addressed the issue of pharmacokinetic interactions with other immunosuppressive agents such as corticosteroids or tacrolimus.

2.3.4. Variability

The global interpatient variability of pharmacokinetic parameters can be split into components that are explained (by individual factors such as the GFR, bodyweight, etc.), or unexplained (unrelated to identified factors). Residual (intrapatient) variability encompasses

biological changes in patients over time, and also imprecision related to drug/sample timing, the analytical assay and imperfect adequacy of the model.

The total variability in oral bioavailability, estimated by pooling published determinations of this parameter, reaches 16%, while interpatient variability assessed in a population pharmacokinetic study in ten transplant recipients amounted to 17% [14]. Food intake before valganciclovir administration was shown to increase ganciclovir exposure by 30% [18], thus the intake modality could explain part of the interpatient or intrapatient variability in oral availability, but no study has formally assessed this question. Variations of less than 20% in oral bioavailability of valganciclovir are not expected to significantly compromise ganciclovir exposure in most patients. Noticeably, ganciclovir bioavailability after valganciclovir administration has not been assessed in lung transplant patients with cystic fibrosis, who are known to have various alterations in drug absorption and disposition.

The total variability in absolute clearance assessed throughout the studies that were reviewed amounted to 36% in transplant patients and 31% in healthy subjects or HIV/AIDS-infected patients. The major part of this variability in clearance was explained by renal function. The studies reporting a correlation with the GFR (an individual approach) suggest that as much as 90% of the variability is explained by kidney function (table 3). In two population studies, the remaining residual intersubject variability in absolute clearance was 25% in a model including only creatinine clearance (n = 10) [13] and 48% in a model including creatinine clearance, bodyweight and the diagnostic group but with important population and assay heterogeneity (n = 53) [46]. The total variability reported in the terminal volume of distribution was 32% in transplant patients and 52% in healthy subjects or HIV/AIDS-infected patients. The unexplained interpatient variability in the central volume of distribution estimated in population studies was 28% (n = 53) [46] and 52% (n = 10) [14], both models including bodyweight. The residual variability reported in those two population studies was 36% [46] and 16% [14].

For oral valganciclovir, the total variability reported in ganciclovir CL/F and the terminal volume of distribution was of the same magnitude as for absolute ganciclovir parameters. The total variability in CL/F and terminal volume of distribution reached 31% and 36% in transplant patients, respectively, and 22% and 29% in healthy subject or HIV/AIDS-infected patients, respectively. Here again, the total variability was mostly explained by the correlation with creatinine clearance. Czock et al. [20] reported a very good correlation with creatinine clearance (measured from a 24-hour urine collection; r^2 = 0.98) (table 3). A population study of both oral ganciclovir and valganciclovir reported an unexplained interpatient variability in ganciclovir apparent clearance of 18% (in a model based on creatinine clearance and bodyweight) [14]. The variability in the apparent central volume of

distribution was partly explained by sex, with the unexplained interpatient variability still amounting to 28% [13] and residual variability to 36% [13].
Thus the variability in pharmacokinetic parameters is fairly similar for intravenous ganciclovir and ganciclovir delivered as oral valganciclovir. The variability in clearance is mainly explained by the GFR and, to a lesser extent, by the patient diagnostic group, while variability in volume of distribution is mainly explained by bodyweight. Beyond those effects, only a moderate amount of unexplained variability remains, making ganciclovir disposition after valganciclovir administration fairly predictable based on a few patient characteristics.

2.4. Pharmacodynamic characteristics

2.4.1. Mechanism of action

Ganciclovir, a nucleoside analogue of endogenous deoxyguanosine triphosphate (dGTP), is effective in vitro against Herpes viruses but is mainly used against CMV infection. Ganciclovir penetrates into cells through purine nucleobase and nucleoside transporters [53,54]. Once inside CMV infected cells, ganciclovir is first phosphorylated by a viral protein kinase and then by host cellular kinases induced during CMV infection into ganciclovir triphosphate. Ganciclovir, activated by phosphorylation into ganciclovir triphosphate, competes with dGTP as a substrate for viral DNA polymerase. Incorporation of ganciclovir triphosphate and reduction of dGTP inclusion into growing chains of viral DNA slows down their extension, thereby inhibiting viral replication [55]. The selectivity of ganciclovir for CMV-infected cells is attributed to weaker inhibition by ganciclovir triphosphate of cellular DNA polymerase compared with viral DNA polymerase, and by the rather specific accumulation of ganciclovir triphosphate in CMV-infected cells [56]. In fact, ganciclovir triphosphate is produced rather specifically in infected cells because ganciclovir has a high affinity for a CMV viral protein kinase, which catalyses its phosphorylation into a monophosphate derivative, while further phosphorylations are catalysed by cellular kinases (figure 1).

2.4.2. Preclinical data

The pharmacodynamic profile of ganciclovir has been widely studied in vitro. The effect of ganciclovir on CMV replication in cell cultures (i.e. human embryonic lung cell cultures) was usually assessed by plaque reduction assays. Antiviral activity is expressed in term of the IC_{50}, defined as the concentration of ganciclovir at which viral replication is inhibited by 50%. The average IC_{50} values are 0.9 mg/l (range 0.1–2.0 mg/l) for the sensitive CMV reference laboratory strain AD169, 0.6 mg/l (range 0.3–1.3 mg/l) for the sensitive clinical reference

strain Towne and 0.7 mg/l (range 0.2–1.9 mg/l) for CMV clinical strains [57-76]. Effective inhibitory concentrations of 0.6–1.6 mg/l were cited in a clinical pharmacodynamic study [77].

Whereas the IC_{50} has been widely determined, there are no data correlating in vitro susceptibility values and in vivo ganciclovir pharmacokinetic parameters such as the AUC, trough plasma concentration (C_{trough}) or time over IC_{50} to predict efficacy. In an experiment on intracellular metabolism of radiolabelled ganciclovir in infected cells, the intracellular level of ganciclovir triphosphate persisted long after the drug was removed from the culture medium (intracellular half-life 6–12 h) [61,78]. The inertia in the formation and disappearance of intracellular phosphorylated derivates suggests that the average ganciclovir exposure, rather than the C_{trough}, is related to clinical efficacy. Resistance of CMV to ganciclovir mostly results from selection of mutations in the viral protein kinase UL97 gene responsible for the monophosphorylation of ganciclovir, and less frequently in the viral DNA polymerase UL54 gene (figure 1). Mutations of the UL97 gene confer resistance to ganciclovir alone, whereas polymerase mutations usually occur in addition to UL97 mutations and may increase the level of drug resistance or confer cross-resistance towards other anti-CMV drugs [55].

Among 300 high-risk, solid organ transplant recipients receiving oral ganciclovir prophylaxis for 100 days, the incidence of CMV UL97 mutation was 2% at the end of prophylaxis and 6% up to 1 year after transplantation (no dual resistance mutations). In contrast, no resistance mutations were detected in the group of patients receiving valganciclovir prophylaxis [79,80].

2.4.3. Prophylactic use

Systemic exposure was correlated with antiviral activity in a population study performed in 240 D+/R- solid organ transplant patients receiving valganciclovir 900 mg once daily or oral ganciclovir 1000 mg three times daily (with the dose adjusted to renal function). A low incidence (1.3%) of breakthrough viraemia during prophylaxis was predicted for an AUC of 50 mg·h/l, whereas this risk was increased up to eight times with an AUC of 25 mg·h/l. One month after discontinuation of prophylaxis, the incidence was estimated to be 10% for patients with an AUC of 50 mg·h/l and 20% for those with an exposition of 33 mg·h/l. However, the risk of developing CMV disease up to 1 year post-transplant was not dependent on ganciclovir exposure during prophylaxis [81].

Regarding CMV antibodies, a meta-analysis found that CMV antiviral prophylaxis prevents CMV disease and all-cause mortality, irrespective of the CMV serostatus. However organ

transplant recipients who are CMV D+/R- are at higher absolute risk of CMV disease than R+ recipients [4]. A retrospective study analysed the efficacy of a risk-stratified dosing regimen in kidney transplant patients (D+/R- recipients received 900 mg once daily and D+/R+ or D-/R+ recipients received 450 mg once daily with adjustment for renal function) without finding any difference between the groups [82]. In this study, ganciclovir plasma concentrations were not monitored.

Five of nine paediatric transplant recipients receiving oral ganciclovir with C_{trough} values greater than a target range of 0.5–1.0 mg/l (based on a previous study in HIV-infected patients under maintenance treatment [83]) did not develop CMV disease, except for one patient whose C_{trough} was not specified [84]. Mean plasma C_{trough} values of 0.9 ± 0.7 mg/l measured in 14 paediatric transplant recipients (D+/R-) receiving oral ganciclovir were effective in preventing CMV disease [85]. This last study targeted a C_{trough} of 0.5–2.0 mg/l, based on C_{trough} values after intravenous ganciclovir treatment in adults (0.2–0.6 mg/l) [28] and IC_{50} values (0.26–1.26 mg/l) [64] but also taking into account that lower peaks were expected with oral ganciclovir. As average ganciclovir exposure seems more predictive of efficacy than the C_{trough}, and considering the difference in the pharmacokinetic profiles of oral ganciclovir and valganciclovir, the C_{trough} reported as being effective after oral ganciclovir cannot be simply extrapolated to oral valganciclovir.

A C_{trough} of 0.3–1.6 mg/l (based on IC_{50} values) was targeted in 68 transplant recipients receiving intravenous ganciclovir for CMV prophylaxis or treatment. Ganciclovir concentrations (peak, trough and mean) in patients who developed CMV infection after prophylaxis or who relapsed after treatment were not statistically different from those in the overall group. However, two D+/R- liver recipients in the prophylaxis group with C_{trough} values <0.3 mg/l subsequently developed CMV disease [77].

A ganciclovir exposure close to 50 mg·h/l seems to be effective to prevent breakthrough viraemia and was not associated with selection of CMV-resistance mutations [79,80]. Whereas no formal C_{trough} cut-off can be confidently defined, C_{trough} values below 0.3 mg/l seem to be insufficient to prevent CMV disease after prophylaxis in D+/R- transplant patients.

2.4.4. Induction treatment

In 15 AIDS patients treated with intravenous ganciclovir for retinitis, 12 had C_{trough} values below 0.6 mg/l, among whom six experienced treatment failure (this cut-off value was chosen based on pharmacokinetic studies reporting C_{trough} values of 0.5–1.3 mg/l associated

with a good response) [38,42]. Four of them improved following a daily dose increase that achieved C_{trough} values over 0.6 mg/l [86].

Eleven paediatric renal transplant recipients under pre-emptive treatment (intravenous ganciclovir for 15 days followed by oral ganciclovir for 3 months) displayed a mean C_{trough} of 1.3 ± 0.8 mg/l and a good virological response. No CMV disease occurred in 10 of 11 patients during the treatment or during the year following discontinuation; however, one D+/R- patient who received an erroneously low oral dose (C_{trough} 0.35 mg/l) relapsed, without a response after an oral dose increase (C_{trough} values of 1.8 and 2.0 mg/l). CMV was found to be resistant to ganciclovir [87].

Ganciclovir is also used to treat symptomatic congenital infection. In 24 babies receiving oral valganciclovir treatment, no correlation was found between ganciclovir pharmacokinetic parameters and changes in the CMV load over the 56 days that CMV was measured in blood [88].

Based on those observations, no clear C_{trough} cut-off can be confidently defined; however, a C_{trough} below 0.6 mg/l seems to be insufficient for some AIDS patients with acute retinitis, while a C_{trough} over 1.3 mg/l seems effective for pre-emptive therapy in transplant patients.

2.4.5. Maintenance treatment

Ganciclovir exposure was predictive of the time to progression of retinitis in AIDS patients receiving maintenance ganciclovir therapy (orally or intravenously). A median time to photographic progression of 50 days was associated with an AUC of 20 mg·h/l. Of note, an important increase in exposure translated into only a moderate increase in the delay to progression. In this analysis, the C_{max} added no significant predictive value to the AUC and the minimum plasma concentration (C_{min}) was not predictive at all, suggesting that total exposure is better correlated with ganciclovir efficacy [31], but this analysis pooled ganciclovir concentrations measured after both oral and intravenous ganciclovir administration.

Mean trough concentrations measured in 14 AIDS patients on maintenance oral ganciclovir therapy were compared between patients with a short interval before recurrence of CMV retinitis (<90 days from the initiation of maintenance therapy) and those with a longer interval (over 90 days). A trend towards lower C_{trough} values in patients with earlier recurrence was reported (0.4 ± 0.3 mg/l vs 0.8 ± 0.6 mg/l). Considering a cut-off of 0.6 mg/l [31,38], the risk of progression was higher in the group of patients with low residual concentrations, although this difference did not reach statistical significance [86].

The time to progression of retinitis seems to be related to the AUC of ganciclovir (over a low range of AUC: 17–31 mg·h/l). No C_{trough} cut-off can be confidently defined; however, C_{trough} values over 0.8 mg/l may better prevent early recurrence of retinitis.

Factors influencing ganciclovir pharmacodynamics were assessed in the pharmacokinetic-pharmacodynamic study by Wiltshire et al. [81] including 240 donor D+/R- solid organ transplant recipients receiving oral ganciclovir or valganciclovir prophylaxis. None of the covariates tested (age, sex, treatment) significantly influenced the correlation between individual exposure to ganciclovir during prophylaxis and the incidence of CMV viraemia during prophylaxis or at 3 weeks after cessation.

2.4.6. Toxicity

Haematological toxicity

The haematological toxicity profile of ganciclovir has been studied in normal human haematopoietic progenitors cells in vitro. The toxicity of ganciclovir was assessed by a clonogenic assay (a technique to determine the effect of the drug on the survival and proliferation of cells). The toxic effect is expressed in terms of the IC_{50}. The IC_{50} was assessed to be 0.7–4.8 mg/l in granulocyte-macrophage progenitors and 0.4–7.4 mg/l in erythroid progenitors [89,90]. Ganciclovir inhibition was dose-dependent in both cell types [89]. In comparison, the ganciclovir cytotoxic concentration for human diploid fibroblasts reached 90 mg/l [61].

Bone marrow suppression was reported in three of five bone marrow transplant recipients receiving intravenous ganciclovir treatment, with mean peak and trough plasma concentrations exceeding 12.8 mg/l and 2.6 mg/l, respectively [69]. However, another study reported neutropenia in bone marrow transplant recipients receiving intravenous ganciclovir, with peak and trough plasma concentrations of 3.9 mg/l and 0.7 mg/l, and found no correlation based on data from 11 patients [39]. Neutropenia occurred in one of six bone marrow transplant recipients treated with intravenous ganciclovir for CVM disease with peak and trough concentrations of 4 mg/l and <0.25 mg/l, respectively, in comparison with 4.8–6.2 mg/l and <0.25–0.6 mg/l reported in the entire group [28]. Neutropenia was reported in 21% of allogeneic haematopoietic stem cell transplant patients (n = 39) after a median of 21 days of pre-emptive therapy with intravenous ganciclovir. No plasma concentrations were reported; however, the total dose was significantly higher in patients who developed neutropenia (P = 0.02). There was a trend towards a higher incidence of neutropenia in patients receiving longer treatment with high-dose ganciclovir (5 mg/kg twice daily for >1

week) [91]. The risk factors for development of neutropenia in allogenic bone marrow transplant patients receiving ganciclovir included elevation of the serum creatinine level [92].

Of 314 immunocompromised patients (262 AIDS patients, 36 transplant recipients and 16 others) with serious CMV infection treated with intravenous ganciclovir, 42% developed neutropenia, 19% thrombocytopenia and 4% anaemia. No relation between the daily dose of ganciclovir and the rate of neutropenia was found [93]. Concomitant medication with zidovudine was reported to exacerbate the haematological toxicity of ganciclovir in patients with AIDS [32].

In solid organ transplant recipients receiving intravenous ganciclovir prophylaxis (n = 44) or treatment (n = 25), the occurrence of neutropenia was also not correlated with the serum concentrations [77]. In 26 transplant patients receiving intravenous ganciclovir treatment, there was no statistically significant relation between the percentage decrease in granulocytes (23% after 15 days) and ganciclovir peak and trough concentrations [25]. In a pharmacokinetic-pharmacodynamic study including 240 D+/R- solid organ transplant recipients receiving oral valganciclovir or oral ganciclovir prophylaxis, analysis of the relationship between exposure and myelotoxicity showed a weak tendency for increased neutropenia and leukopenia but not anaemia and high exposure. Median incidences of neutropenia of 15% and 20% were reported with AUC values of 39 mg·h/l and 61 mg·h/l, respectively. For leukopenia, exposition of 34 mg·h/l and 62 mg·h/l were associated with incidences of 40% and 50%, respectively [81]. However, this study did not address the contribution to haematotoxicity of concomitant medications (i.e. mycophenolate mofetil, azathioprine and cotrimoxazole, etc.).

Globally, the incidence of neutropenia is higher in bone marrow transplant patients and in patients with advanced HIV infection than in solid organ transplant patients [92]. Ganciclovir inhibition of DNA-polymerase in haematopoietic progenitor cells has been shown to be dose-dependent [89]; however, only a few studies have demonstrated a relation between the ganciclovir concentration and this adverse event. Thus no toxic interval can be confidently defined. Other factors may confound this issue, such as CMV disease itself or concomitant medications (i.e. cotrimoxazole, mycophenolate mofetil, azathioprine, etc.), which can also induce neutropenia.

The incidence of agranulocytosis during valganciclovir prophylaxis reaches 5–6%; however, neutropenia-associated sepsis remains fairly rare [5,94]. This adverse effect seems to be delayed and is transient once valganciclovir and mycophenolate mofetil are discontinued or interrupted [94].

Neutrophil and leukocyte counts should be monitored in patients receiving high doses of ganciclovir or valganciclovir over long periods, especially bone marrow transplant patients or

patients receiving others drugs with haematological toxicity. No monitoring frequency has been defined, but it is to be recalled that a 2-week lag precedes the haematological expression of myelotoxicity of most agents. In the case of agranulocytosis, discontinuation of valganciclovir, mycophenolate mofetil and cotrimoxazole is mandatory.

Neurotoxicity

Neurotoxicity has been described in seven case reports, after 2 days to 1 month of therapy, with a probable or possible likelihood of ganciclovir-induced toxicity [94-101]. Symptoms (agitation, confusion, hallucination, disorientation) decreased or disappeared after dose reduction or drug withdrawal, and three case reports described a positive rechallenge [97-99]. Seizures were associated with intravenous ganciclovir therapy with a positive rechallenge in a patient with AIDS and disseminated CMV infection [99]. Cerebrospinal fluid (CSF) and plasma trough concentrations of ganciclovir measured 3 days after drug withdrawal were 0.75 mg/l and 1.2 mg/l, respectively, in a patient undergoing maintenance haemodialysis and receiving intravenous ganciclovir 1.25 mg/kg once daily [95]. This CSF concentration was higher than those described 3–6 h after administration of ganciclovir 2.5 mg/kg in patients with normal renal function [28]. The treatment was well tolerated after a dose reduction to 1.25 mg/kg after each haemodialyis session. CSF and plasma ganciclovir concentrations 48 h after the last dose of valganciclovir in a child with impaired renal function (GFR 20 ml/min) receiving 450 mg every other day were 2.6 and 3.9 mg/l, respectively. Tests for CMV and Herpes virus in the CSF remained negative. Further, the same valganciclovir dosage was well tolerated (with a ganciclovir concentration at 24 h of 2.9 mg/l) [96].
CNS adverse effects were reported in a renal transplant patient with mild renal insufficiency with a ganciclovir C_{trough} of 2.0 mg/l after intravenous ganciclovir 5 mg/kg twice daily. The patient improved 3 days after a dose reduction to 1.25 mg/kg every 12 h [94]. Neurotoxicity has been reported mainly in patients with renal insufficiency but also in a patient with normal renal function receiving intravenous ganciclovir with a positive rechallenge, but no plasma concentrations of ganciclovir were reported in this last case [98].
Thus neurotoxicity seems to be related to high plasma (and CSF) concentrations, but no toxic range can be deduced on the basis of these reports. Patients with renal failure whose dosage is not adapted seem to be at higher risk of developing neurotoxicity.

Hepatotoxicity

Hepatic damage (mild to severe) was reported in 5 of 14 courses of intravenous ganciclovir therapy in 11 patients with AIDS, with drug imputability estimated as being possible [102].

One patient with AIDS and CMV retinitis showed marked elevations in transaminases and alkaline phosphatases during treatment with ganciclovir 2.5 mg/kg three times daily and once again when receiving a reduced dosage (1.5 mg/kg every 8 h). Ganciclovir was estimated as most likely responsible for this toxicity (no other toxic or infectious reasons were incriminated) [103].

Ganciclovir appears to induce hepatotoxic effects in isolated transplant recipients; however, no information is available about a potential relation between the drug concentration and toxic effects.

2.5. Assessment of evidence justifying therapeutic drug monitoring for valganciclovir

The issue of the relevance of TDM of valganciclovir has not been addressed. However, routine TDM of ganciclovir has been evaluated as being unwarranted in solid organ transplant patients [9].

Based on this review of the pharmacokinetic and pharmacodynamic characteristics of valganciclovir, the following answers can be given to the classical questions addressing the potential usefulness of valganciclovir TDM in solid organ transplant patients [104].

(i) *Is the patient on the best drug for his specific subpopulation (disease state) and specific indication?* Valganciclovir has been shown to be successful for CMV prophylaxis in D+/R- solid organ transplant patients and is commonly used for prophylaxis and treatment of CMV infection, with clinical responses at least equivalent to those observed with oral or intravenous ganciclovir, and greater convenience [105-115].

(ii) *Can the drug be readily measured in the desired biological matrix?* Ganciclovir, rather than valganciclovir, should be measured, as valganciclovir is a prodrug of ganciclovir. A number of analytical methods have been developed and validated for measurement of ganciclovir in biological fluids, usually by high-performance liquid chromatography with UV or fluorimetric detection [116]. Ganciclovir is activated in the infected cells, thus measurement of intracellular concentrations of phosphorylated derivates might be more informative than measurement of serum or plasma concentrations. However, it would be technically demanding to develop such an assay for routine clinical practice, and it would be meaningful only in infected cells, which are not available in clinical specimens.

(iii) *Has a good correlation between the drug concentration and the pharmacological response been reported in pharmacokinetic studies conducted in humans?* The inhibition of CMV viral replication is dose dependent in vitro. A statistically significant relation has been reported between ganciclovir exposure and the efficacy of prophylaxis in transplant recipients and also with time progression of retinitis in patients with AIDS receiving maintenance oral ganciclovir therapy. Five studies in small groups of patients (AIDS patients or transplant recipients) showed a global trend towards a higher incidence of treatment failure (for prophylactic or therapeutic use) in patients with low ganciclovir C_{trough} values. However, such associations are rather loose, and no target concentration has been formally identified to date.

(iv) Is the pharmacological response of the drug not readily assessable? Ganciclovir inhibits CMV viral replication by inhibition of viral DNA synthesis. Viraemia can be measured by different methods (i.e. CMV DNA copies/ml by polymerase chain reaction). However, the CMV DNA load decreases usually by about -1.0 log of copies/ml per week during induction treatment, and in some patients, a decrease is only observed after a longer period. Thus the pharmacological response is only roughly assessable with a certain delay. The response to prophylactic treatment is not assessable at all.

(v) Does the correlation between the concentration and the pharmacological response still apply to the patient's specific subpopulation (disease state) and specific indication? There has been one study revealing a statistically significant relation between ganciclovir exposure and the efficacy of prophylaxis in transplant recipients. However, three studies in small groups of patients showed a global trend towards a higher incidence of treatment failure in patients with low ganciclovir C_{trough} values.

(vi) Does the drug have a narrow range for the specific population (disease state) and specific indication? No toxic range can be confidently established on the basis of current knowledge, but a tendency towards an increased occurrence of adverse events was reported with elevated ganciclovir exposure. Only one study has suggested a therapeutic range for CMV prophylaxis (AUC 40–50 mg·h/l defined on the basis of 240 transplant recipients at high risk of CMV infection) [81]. For CMV treatment, only studies in small groups of transplant patients have been conducted, revealing a trend towards treatment failure in patients with low ganciclovir plasma concentrations, but no therapeutic range could be defined. In vitro, however, the ganciclovir IC_{50} for replication of sensitive CMV viral strain and for colony formation

of human haematopoietic progenitors are very close, suggesting a narrow range between therapeutic efficacy and haematological toxicity

(vii) Are the pharmacokinetic parameters unpredictable because of either intrinsic variability or the presence of others confounding factors? The pharmacokinetic parameters of valganciclovir have been determined in kidney, kidney-pancreas, liver and cardiac transplant recipients, revealing fairly reproducible oral availability, an absolute clearance highly correlated to the GFR, and a central volume of distribution correlated to bodyweight and sex. Unexplained variability is rather moderate.

(viii) Is the duration of drug therapy of a sufficient length for the patient to benefit from TDM? Induction treatment for CMV infection/disease is administered for at least 2 weeks and followed by maintenance treatment (or secondary prophylaxis) for at least 1 month. The duration of CMV prophylaxis is usually between 3 and 6 months, which obviously leaves time for dosage adjustment to reveal benefit.

(ix) Will the results of the drug assay make a significant difference in the clinical decision-making process (i.e. provide more information than sound clinical judgement alone)? In some situations, clinical judgement may not be sufficient; for instance, if the degree of renal insufficiency is difficult to assess, if the clinical condition of the patient changes (renal impairment, oedema), if there is no decrease in the viral load during treatment of CMV infection, if there is heavy concomitant medication use in a patient showing signs of toxicity, if comorbidity severely interferes with gastrointestinal absorption, etc.

Based on those criteria, routine use of TDM for valganciclovir dosage adaptation in solid organ transplant recipients does not appear to be indicated, although adjustment for renal function and possibly bodyweight is recommended. Dosage adaptation to bodyweight is not advised by the manufacturer but may make sense in markedly underweight or overweight patients, as bodyweight has been reported in several studies to impact not only on the volume of distribution (without a consequence in terms of the AUC), but also on clearance (table 3). Thus in patients weighing 40 kg versus 70 kg with similar renal function, half the usual dosage may be sufficient to ensure roughly similar exposure [13]. Conversely, a patient weighing 100 kg could be underexposed with the usual dosage and may benefit from better coverage with 50% higher maintenance doses.

However, current knowledge poorly covers certain patient populations (e.g. patients with lung transplants, cystic fibrosis or malabsorption). Thus TDM may be useful in specific situations. It may help to define the lowest efficacious dosages for prophylactic use. It may document exposure in unusual conditions (e.g. serious digestive disease or continuous renal

replacement therapy). More studies are needed to delineate indications for requesting valganciclovir TDM for optimal patient management. Finally, TDM may provide useful information in clinical trials of valganciclovir.

2.6. How to translate a concentration result into an advice on dosage adjustment?

The pharmacokinetic parameters of valganciclovir (bioavailability, apparent clearance and the volume of distribution) in solid organ transplant recipients appear fairly predictable based on the knowledge of significant patient characteristics. Thus the ganciclovir concentration profile can theoretically be estimated from the patient's GFR and bodyweight, in addition to the dosing regimen. In practice, when faced with a test result, the clinical pharmacologist should first check its consistency with the theoretical prediction. A marked discrepancy should prompt the search for its source (e.g. a problem of adherence, malabsorption, drug interaction, or any yet unidentified factor). A formal Bayesian framework for TDM-based dosage adjustment may then be employed. Alternatively, a simple comparison of the observed concentration with the therapeutic range usually achieved in CMV prophylaxis or treatment may be sufficient to work out an appropriate dosage adjustment. It should be recalled that therapeutic target concentrations are poorly defined for ganciclovir. Average exposure appears to be better correlated with efficacy than C_{trough} values. Bayesian-derived pharmacokinetic parameter values may be obtained from a single measurement based on the results of a previous population pharmacokinetic study, considering the fair predictability of valganciclovir disposition. However, there have been few studies to provide robust Bayesian priors and to define therapeutic or toxic intervals. For the first approximation, for CMV prophylaxis, an AUC value between 40 and 50 mg·h/l seems to be related to effective prevention of breakthrough viraemia in transplant recipients at high risk of CMV infection [81]. Considering the regularity of ganciclovir pharmacokinetics after valganciclovir administration, and the fact that most of its variability is in the clearance, the AUC is expected to be fairly predictable from a single C_{trough} measurement. To date, however, no study has reported correlations between the AUC and C_{min}. For pre-emptive CMV treatment in transplant patients, C_{trough} values >1.3 mg/l were related to efficient therapy [87]. This cut-off value also corresponds to concentrations observed in liver transplant recipients 12 h after a dose of intravenous ganciclovir 5mg/kg or oral valganciclovir 900 mg given twice daily [10]. As indicated above, preventive use of valganciclovir may target approximately half those concentration levels.

2.7. Conclusion

The pharmacokinetic parameters of valganciclovir are fairly predictable in solid organ transplant recipients whose GFR and bodyweight are known. Unexplained interpatient variability is limited, suggesting no major effect of undefined parameters. Bioavailability appears to be reproducible, and no problem of absorption has been reported in solid organ transplant patients; however, no data are available in patients with lung transplants, severe malabsorption or cystic fibrosis. Conversely, the pharmacodynamic properties of valganciclovir and ganciclovir are not characterized with sufficient precision to define formal therapeutic or toxic intervals. Nevertheless, in vitro activity is reproducible against sensitive viral strains with an IC_{50} close to 1 mg/l.

The contribution of routine TDM of valganciclovir to management of solid organ transplant recipients therefore appears questionable, compared with a dosage adjustment strategy based only on the patient's GFR, bodyweight and possibly the indication for treatment (AIDS-related versus transplant related anti-CMV treatment). However, ganciclovir measurement may still be useful in particular cases, either with factors that have an unknown impact on valganciclovir absorption and disposition, or with an unexplained lack of efficacy or with toxicity.

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Table 1: Pharmacokinetic characteristics of ganciclovir after oral valganciclovir administration[a]

Patient population (no. of patients)	**BW** (kg)	**GFR** (m/min)	**Dose** (mg)/ Interval(h)	**No. of samples**	**F** (%)	**C_{max}** (mg/l)	**t_{max}** (h)	**C_{min}** (mg/l)	**AUC** (mg·h/l)	**CL/F** (l/h)	**$t_{½}$** (h)	**V_z/F** (l)	**REF**
LiT (28)	88 ± 18	93 ± 21	450	16	60	3,0 ± 0,8	3,0 ± 1,3	*0.15*[b]	22,2 ± 5,3	*14,6 ± 3,5*[c]	5,2 ± 1,0	*110 ± 34*[d]	10
	88 ± 18	93 ± 21	900	16	59	6,2 ± 1,9	2,9 ± 1,0	*0.3*[b]	43,9 ± 11,0	*14,8 ± 3,7*[c]	5,1 ± 1,1	*109 ± 34*[d]	
KT (6)	70[e]	>60	450/24	10	-	4.1[f]	2[f]	0.3[f]	35.9[f]	*9,3*[c]	-	-	11
KT (7)	70[e]	>60	900/24	10	68	8.0[f]	1.8[f]	0.5[f]	62.9[f]	*10,2*[c]	-	-	
KT (21)	88 ± 23	>60	900/24	12	-	6,9 ± 1,5	3,0[f]	*0.4*[b]	52,2 ± 10	*12,9 ± 2,7*[c]	5,7 ± 1,4	*105 ± 30*[d]	12
	88 ± 23	>60	900/24[g]	12	-	6,6 ± 1,8	2,0[f]	*0.4*[b]	52,3 ± 10,3	*12,9 ± 2,8*[c]	5,7 ± 1,3	*105 ± 26*[d]	
LiT, KT, HT, KPT (160)	80 ± 21	91 ± 36	900/24[h]	3 (x2)	-	-	-	-	46,3 ± 15,2[i]	12,4[j]	6,3[k]	113[k]	5,13
LiT, KT, HT (10)	65	-	900/12	-	88 7	-	-	-	-	-	-	-	14
SCT[l] (22)	76 [52-107][m]	92 ± 45	900	10	-	6,7 ± 1,8	3,5 ± 0,9	*<1*[b]	52,1 ± 21,3	*12,4 ± 4,0*[c]	5,1 ± 1,4	*92 ± 45*[d]	15
SCT (22)	72 ± 12	>25	900/12[h]	9	76 ± 18	8,8 ± 2,4	2,7 ± 0,8	1,7±0,9	53,8 ± 18,0	*12,0 ± 4,0*[c]	4,2 ± 1,1	*73 ± 31*[d]	16
HIV (17)	74 ± 10	>70	360[n]	16	61 ± 9	3,0 ± 0,8	1,0 ± 0,3	*0.2*[o]	10,8 ± 1,9	*24,0 ± 4,2*[c]	3,7 ± 0,6	*128 ± 31*[d]	17
HIV (16)	76 [60-97][m]	114 [77-155][m]	450/24[p]	17	-	3,1 ± 0,5	1[f]	*0.5*[o]	10,3 ± 2,6	*31,5 ± 7,9*[c]	3,9 ± 0,8	*178 ± 57*[d]	18
	76 [60-97][m]	114 [77-155][m]	875/24[p]		-	5,3 ± 1,1	1.5[f]	*0.1*[b]	19,0 ± 3,8	*33,2 ± 6,6*[c]	4,1 ± 0,7	*196 ± 50*[d]	
HIV (16)	73 [58-95][m]	116 [81-154][m]	450/24[q]	17	-	3,3 ± 1,1	1.5[f]	*0.5*[o]	12,7 ± 1,9[r]	*25,5 ± 3,8*[c]	3,8 ± 0,8	*140 ± 37*[d]	
	73 [58-95][m]	116 [81-154][m]	875/24[q]		-	6,1 ± 1,7	1.5[f]	*0.1*[b]	24,8 ± 3,7[r]	*25,4 ± 3,8*[c]	4,1 ± 0,7	*150 ± 34*[d]	
AIDS (20)	70[d]	>7	900/24	10	59	5,9 ± 1,8	2.0[f]	*0.15*[b]	34,9 ± 10,3	*18,6 ± 7,1*[c]	4,1 ± 0,9	*110 ± 48*[d]	19
AIDS (25)	70[d]	>70	900/12	10	64	6,7 ± 2,1	2.0[f]	*1*[b]	32,8 ± 10,1	*19,8 ± 6,1*[c]	3,9 ± 1,1	*111 ± 47*[d]	
HS (8)	74 ± 14	93 ± 16	900	8	59 ± 7	5,8 ± 1,7	2,0 ± 1,0	*<0.3*[b]	28,1 ± 5,8	24,1 ± 5,8	3,5 ± 0,8	121 ± 29	20
HIV (8)	75 ± 7	104 ± 17	900	8	61 ± 5	5,7 ± 1,1	1,9 ± 0,4	-	27,1 ± 3,5	24,2 ± 3,1	3,8 ± 0,5	134 ± 27	
mild RI (6)	68 ± 6	61 ± 6	900	9	-	6,9 ± 2,5	2,2 ± 1,0	-	50,5 ± 23,0	14,9 ± 5,9	4,9 ± 1,4	96 ± 21	20
medium RI (6)	79 ± 9	39 ± 10	900	9	-	7,1 ± 1,6	3,0 ± 1,1	-	100 ± 54	8,2 ± 3,8	10 ± 4,4	101 ± 15	
severe RI (6)	74 ± 4	13 ± 2	900	9	-	8,5 ± 1,2	4,3 ± 3,9	-	252 ± 64	2,7 ± 0,7	22 ± 5,2	82 ± 14	

Legend of table 1:

[a] values are expressed as mean ± SD unless specified otherwise.
[b] determined graphically.
[c] calculated as dose(mg) /AUC(mg·h/l).
[d] calculated as [CL/F(l/h)·t ½ (h)]/ln(2).
[e] standard body weight (no body weight mentioned).
[f] median.
[g] tutti-fruitti-flavored oral valganciclovir solution.
[h] dose adjusted for the GFR.
[i] geometric mean.
[j] estimate with a combined pharmacokinetic model for ganciclovir delivered by oral ganciclovir and valganciclovir in a 80 kg patient with a GFR of 80 ml/min.
[k] calculated from systemic and inter-tissue clearance and from central and peripheral volumes of distribution.
[l] stable graft-versus-host disease of the gastrointestinal tract.
[m] oral aqueous solution of 30 mg/mL.
[n] determined graphically after extrapolation.
[o] fasted.
[p] fed.
[q] the effect of food on the AUC_{24} as statistically significant (P<0.001); in fed stage, the AUC_{24} increased proportionally with the dose.

AUC = area under the plasma concentration-time curve; **AUC_{24}** = AUC from 0 to 24 h; **AIDS** = patients with acquired immunodeficiency disease; **BW** = bodyweight; **CL/F** = apparent oral clearance; **C_{max}** = maximum plasma concentration; **C_{min}** = minimum plasma concentration; **F** = oral bioavailability; **GFR** = glomerular filtration rate; **HIV** = HIV-seropositive patients, **HS** = healthy subjects; **HT** = heart transplant recipients; **KPT** = kidney-pancreas transplant recipients; **KT** = kidney transplant recipients; **LiT** = liver transplant recipients; **REF** = reference; **RI** = patients with renal insufficiency; **SCT** = stem cell transplant recipients; **t_{max}** = time to reach the C_{max}; **$t_{½}$** = elimination half-life; **V_z/F** = terminal volume of distribution after oral administration.

Table 2 : Pharmacokinetic characteristics of intravenous ganciclovir[a] (first part)

Patient population (no. of patients)	**BW** (kg)	**GFR** (ml/min)	**Dose** (mg/kg)/ **interval** (h)	**No. of samples**	C_{max} (mg/l)	t_{max} (h)	C_{min} (mg/l)	**AUC** (mg·h/l)	**CL** (l/h)	$t_{½}$ (h)	V_z (l)	**REF**
LiT (27)	88 ± 18	93 ± 21	5	16	12.2 ± 2.9	1.0 ± 0.1	0.3[b]	50.6 ± 20.2	9.4 ± 2.6	5.2 ± 1.1	52 ± 13[c]	10
LiT, KT (9)	72 ± 13	61 ± 13	200[d]	11	-	-	-	-	7.6 ± 2.7[e]	5.9 ± 1.6	-	21
KT (6)	70[f]	> 70	2.5/12	10	6.8[g]	1[g]	0.9[g]	60.6[g]	5.8	-	-	11
KT (12)	79 ± 19	68 ± 18	5	10	10[b]	-	0.5[b]	-	8.5 ± 3.4[e]	5.4 ± 1.2	54 ± 15[c,h]	22
KT (32)	70[f]	-	5/12[i]	11	9.3 ± 0.3	-	-	37.4 ± 2.3	7.7 ± 0.4[h]	3.4 ± 0.3	34 ± 1[h]	23
KT (5)	67 ± 7	53 ± 15	5/24[i]	4	8.4 ± 1.9	-	0.5 ± 0.4	49.7 ± 10.1	6.4 ± 2.7	6.7 ± 2.5	55 ± 6	24
T (26)	-	88 ± 44[j]	8.8 ± 3.3	-	9.4 ± 4.2	-	1.7 ± 1.5	-	-	-	-	25
SCT[k] (22)	76 [52-107]	92 ± 45	5	10	13.3 ± 4.0	0.9 ± 0.1	<0.5[b]	53.8 ± 21.5	7.1 ± 2.8[l]	5.2 ± 1.5	53 ± 26[m]	15
SCT (22)	72 ± 12	>25	5/12[i]	9	10.3 ± 2.1	1.1 ± 0.6	1.0 ± 0.7	39.5 ± 13.9	9.1 ± 3.5[l]	3.4 ± 0.8	45 ± 20[m]	16
SCT, BMT (5)	70[f]	>70	200[e]	-	6.0 ± 1.8	1.1	-	29.2 ± 14	8.0 ± 3.4	6.0 ± 1.5	50 ± 15[c]	26
BMT (5)	70[f]	normal	2.5/8	12	5.4 ± 0.7	-	1.1 ± 0.3	-	-	3.6 ± 1.4	-	27
BMT (5)	70[f]	normal	5/8	12	12.4 ± 5.5	-	2.9 ± 2.2	-	-	3.6 ± 1.4	-	
BMT, AIDS (6)	53 ± 10	80 ± 31	2.5/8	7	4.2 ± 0.3	-	0.4 ± 0.2	-	11.2 ± 5.7	2.5 ± 1.1	36 ± 15	28
HS (8)	74 ± 14	93 ± 16	5	8	9.0 ± 1.3	-	< 0.3[b]	25.4 ± 4.3	14.3 ± 2.3	3.3 ± 0.5	68 ± 15	20
HIV (8)	75 ± 7	104 ± 17	5	8	9.6 ± 1.1	-	-	25.4 ± 3.7	15.1 ± 1.7	3.2 ± 0.4	68 ± 4	
HIV (17)	75 ± 10	>70	5	16	9.4 ± 0.8	0.9 ± 0.1	0.3[b]	25 ± 3.8	15.2 ± 3.1[e]	3.7 ± 0.6	79 ± 15[h]	17
HIV (18)	70[f]	normal	5	13	8.3 ± 1.0	1.0 ± 0.1	<0.05	22.1 ± 3.2	16.2 ± 2.5[e]	3.3 ± 0.3	76 ± 12[h]	29
HIV (16)	77 ± 13	> 70	5	11	9 ± 1.4		<0.2	26.8 ± 6.1	14.9 ± 3.8[e]	3.5 ± 0.5	54 ± 11[c,h]	30
HIV (57)	70[f]	> 50	5/24[i]	-	11	-	0.09	30.7	11.4[l]	-	-	31
HIV (22)	70[f]	no gross anomaly	5/12	15	-	-	0.4[b]	-	-	3.3 ± 0.7	109 ± 110[c]	32

Table 2 : Pharmacokinetic characteristics of intravenous ganciclovir[a] (second part)

Patient Population (no. of patients)	**BW** (kg)	**GFR** (ml/min)	**Dose** (mg/kg)/ **Interval** (h)	**No. of samples**	C_{max} (mg/l)	t_{max} (h)	C_{min} (mg/l)	**AUC** (mg·h/l)	**CL** (l/h)	$t_{½}$ (h)	V_z (l)	**REF**
AIDS (18)	70[f]	> 70	5/24	10	9.9 ± 3.1	1.0[g]	0.08[b]	30.7 ± 7.7	11.4 ± 2.9[l]	4.3 ± 0.7	71 ± 21[m]	19
	70[f]	> 70	5/12	10	10.4 ± 4.9	1.0[g]	0.6[b]	28.6 ± 9	12.2 ± 3.9[l]	4.0 ± 0.9	70 ± 27[m]	
AIDS (15)	-	> 70	5/12	2	7.2 ± 2.4	-	0.6 ± 0.3	-	-	-	-	33
AIDS (6)	58 ± 2	no gross anomaly	1/8	-	1.8 ± 0.4	-	0.2 ± 0.1	-	-	-	-	34
AIDS (16)	55 ± 10	no gross anomaly	2.5/8	-	4.9 ± 1.0	-	0.5 ± 0.3	-	-	-	-	
AIDS (20)	70[f]	101 ± 29[i]	2.5/8	-	5.2 ± 1.2	-	0.7 ± 0.5	-	10.2 ± 4.1[j]	4.2 ± 1.6	50 ± 11[c,h]	35
AIDS (6)	68 ± 8	91 ± 23	3.75	11	6.1 ± 1.2	-	0.04[b]	-	14.1 ± 6.7[e]	4.5 ± 2.4	58 ± 22[c,h]	36
AIDS (7)	62 ± 13	82 ± 22	6	11	6.6 ± 1.8	-	0.05[b]	-	19.5 ± 7.2[e]	4.0 ± 1.1	79 ± 26[c,h]	
AIDS (4)	70[f]	normal	2.5/8	9	4.6	-	0.5	45.9[o]	11.4 [d]	-	-	37
	70[f]	normal	5/24	9	10.2	-	0.05	30.6	11.4 [d]	-	-	
BMT, AIDS[p] (8)	-	normal	5/12	2	11.5 [4.8-24.1][n]	-	1.4 [0.1-3.5][n]	-	-	-	-	38
BMT, AIDS[q] (22)	-	-	2.5/8[i]	-	4.1 [1.7-7.8][n]	-	0.61 [0.02-1.7][n]	-	-	-	-	39
CMV[r](51)	70[f]	-	5/24	-	-	-	-	-	12.2 ± 4.6[j]	3.5 ± 1.4	45 ± 15[j]	40
SCT (7)	58 [37-80][g]	98 [75-142][g]	5	8	9.2[g]	-	0.6 [0.2-2.9][g]	29.8 [20.2-111][g]	10.5[e]	3.6 [3.4-7.9][g]	54[m]	41
BMT, AIDS (12)	63 ± 13	137 ± 80	5	11	5.7 ± 1.6	-	-	27.5 ± 18.5	13.8 ± 6.4	3.6 ± 1.4	67 ± 26	42
SCT(5)	58 [37-80][g]	59 [51-67][g]	2.5	8	4.75[g]	-	0.6 [0.4-0.8][g]	24.6 [22.5-28.3] [g]	5.7[e]	5.8 [5.1-8.9][g]	48[m]	41
AIDS, LiT[q] (7)	66 ± 6	41 ± 22	5	11	-	-	-	95.7 ± 49.5	4.7 ± 3.3	11.5 ± 3.9	68 ± 28	42
HT (11)	70[f]	mild RI	2.5/12	15	5.4 ± 1.2	-	1.2 ± 0.6	34.1 ± 15.6	5.1 ± 2.3[l]			43
HT (14)	70[f]	mild RI	5/24	15	11.0 ± 2.7	-	1.2 ± 1.1	78.8 ± 43.2	4.4 ± 2.4[l]			

Legend of table 2:

[a] values are expressed as mean ± SD unless specified otherwise.
[b] determined graphically.
[c] V_{ss}.
[d] dose (mg).
[e] calculated as CL(l/h·kg)*BW_{mean}(kg).
[f] standard BW(no BW mentioned).
[f] median [range].
[h] calculated as V_d(l/kg)* BW_{mean}(kg).
[i] dose adjusted for kidney function.
[j] per 1.73m^2.
[k] stable graft-versus-host disease of the gastrointestinal tract.
[l] calculated as dose(mg/kg)/AUC(mg·h/l) · BW_{mean}(kg).
[m] calculated as [CL/F(l/h/kg) $t_{½}$(h)]/ln(2) · BW_{mean}(kg).
[n] mean [range].
[o] AUC_{0-24}.
[p] plus LiT, KT.
[q] plus KT, HT.
[r] patients with life-threatening CMV disease without any other information.

AIDS = patients with acquired immunodeficiency disease; **AUC** = area under the plasma concentration-time curve; **AUC_{24}** =AUC from 0 to 24 h; **BMT**= bone marrow transplant recipients; **BW** = bodyweight; **CL**= apparent total body clearance; **C_{max}** =maximum plasma concentration; **C_{min}** = minimum plasma concentration; **CMV** = cytomegalovirus; **GFR** = glomerular filtration rate; **HIV** = HIV seropositive patients; **HS** = healthy subjects; **HT** = heart transplant recipients; **KT**= kidney transplant recipients; **LiT** = liver transplant recipients; **RI** = patients with renal insufficiency; **SCT**= stem cell transplant recipients; **T** = transplant recipients; **t_{max}** = time to reach C_{max}; **$t_{½}$** = elimination half-life; **V_d** = volume of distribution; **V_{ss}** = volume of distribution at steady state; **Vz** = terminal volume of distribution.

Table 3: Correlation between clearance, volume of distribution and patient characteristics

Patient population (no. of patients)	Drug	Correlation[a]	r^2	Reference
HS, HIV, RI (38)	oral VGC	$CL/F = 3.6\ [3.2\text{-}4.0]^{b} \times GFR + 0.52$	0.98	20
LiT, KT, HT, KPT (160)	oral VGC	$CL/F = 12.4\ [11.8\text{-}13.0]^{b} \times (GFR/80)^{0.95} \times (BW/80)^{0.73\ c}$ $V_1/F = 25.0\ [19.2\text{-}30.8]^{b} \times EXP^{(-0.53 \times Sex)\ d}$		13
LiT, KT, HT (10)	oral VGC IV GCV	$CL = 2.5 \pm 0.2 \times GFR$ $V_1 = 34.3 \pm 5.3 \times (BW/65)$		14
KT (10)	IV GCV	$CL = 1.8 \times GFR + 1.52$	0.8	22
KT[e] (4)	IV GCV	$CL = 2.7 \times GFR - 2.8$	0.86	24
AIDS[f] (20)	IV GCV	$CL = 1.8 \times GFR$		35
AIDS, BMT, KT, LiT, HT[f] (10)	IV GCV	$CL = 1.25 \times GFR + 0.08$	0.92	42
BMT, AIDS[f] (6)	IV GCV	$CL = 2.4 \times GFR$		28
TR (5) HIV (CMV retinitis) (31) HIV (CMV-infected)[g] (17) TR, HIV[h] (53) TR, HIV[h] (53)	IV GCV IV GCV IV GCV IV GCV IV GCV	$CL = 0.04 \pm 0.006 \times (GFR/6) \times BW + 0.38$ $CL = 0.24 \pm 0.08 \times (GFR/6) \times BW + 0.38$ $CL = 0.17 \pm 0.02 \times (GFR/6) \times BW + 0.38$ $V_1 = 0.39 \pm 0.03 \times BW$ $V_2 = 0.51 \pm 0.03 \times BW$		46
HIV[i] (43)	IV GCV	$CL = 0.22 \pm 0.084 \times (GFR/6) \times BW$		47

Legend of table 3:

[a] CL/F, CL and GFR expressed in l/h; BW expressed in kg; V_1, V_1/F and V_2 expressed in l.
[b] the values in square brackets denote the 95% confidence interval.
[c] rounded median GFR and BW values deduced from the article.
[d] with sex = 1 for female, sex = 0 for male.
[e] linear regression with individual value of creatinine clearance calculated from the Cockroft-Gault equation.
[f] normalized for body surface area.
[g] HIV patients shedding CMV into urine but without retinitis.
[h] Combined population: transplant patients and HIV-patients.
[i] Same population of HIV patients as that studied by Yuen et al.[46]

BMT = bone marrow transplant recipients; **BW** = bodyweight; **CL** = apparent total body clearance; **CL/F** = apparent oral clearance; **CMV** = cytomegalovirus; **GFR** = glomerular filtration rate; **HIV** = HIV-seropositive patients; **HS** = healthy subjects; **HT** = heart transplant recipients; **IV** = intravenous; **KPT**= kidney-pancreas transplant recipients; **KT** = kidney transplant recipients; **LiT** = liver transplant recipients; **RI** = patients with renal insufficiency; **TR** = solid-organ transplant recipients with renal dysfunction; **V_1** = central volume of distribution; **V_1/F** = central volume of distribution after oral administration; **V_2** = peripheral volume of distribution.

Chapter 3 : Analytical method validation

Presentation of chapter 3

Now we have reviewed the current knowledge of the PK and PD of valganciclovir. This will help us to elaborate a clinical investigation completing our appraisal of valganciclovir characteristics in our patients. But before this, an analytical method has to be developed. What will be its performances, precision and reproducibility?

The following chapter is an article describing the analytical method for the measurement of ganciclovir plasma concentrations developed for this work.

During this development, the method is built up and optimized. Once satisfactory separation has been obtained, an important and time-consuming validation process is necessary to ensure precise and unbiased concentration results. Indeed, significant errors on plasma concentrations would bias the results of the pharmacokinetic study. Thus, the validation of the analytical method is as important as the data analysis itself.

Determination of aciclovir and ganciclovir in human plasma by liquid chromatography – spectrofluorimetric detection and stability studies in blood samples

N. Perrottet[1], A. Beguin[1], P. Meylan[2], M. Pascual[3], O. Manuel[3], T. Buclin[1], J. Biollaz[1] and L.A. Decosterd[1]

[1] Division de Pharmacologie clinique, Département de Médecine,
[2] Institut de Microbiologie,
[3] Centre de Transplantation d'Organes,
Centre Hospitalier Universitaire Vaudois, Lausanne,
Switzerland

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Related appendices: 1.1 and 1.2

3. Determination of aciclovir and ganciclovir in human plasma by liquid chromatography – spectrofluorimetric detection and stability studies in blood samples

3.1. Abstract

A sensitive HPLC method has been developed for the assay of aciclovir and ganciclovir in human plasma, by HPLC coupled with spectrofluorimetric detection. Plasma (1000 μl), with 9-ethyl-guanine added as internal standard, is submitted to protein precipitation with trichloroacetic acid solution 20%. The supernatant, evaporated to dryness at 37°C, is reconstituted in 100 µl of a solution of sodium heptanosulfonate 0.4% adjusted with acetic acid to pH 2.60 and a 30 μl volume is then injected onto a Nucleosil 100-5 µm C18 column. Aciclovir and ganciclovir are analysed by spectrofluorimetric detection set at 260 nm (excitation) and 380 nm (emission) using a gradient elution program with solvents constituted of acetonitrile and a solution of sodium heptanosulfonate 0.4% adjusted to pH 2.60. The calibration curves are linear between 0.1 and 10 μg/ml. The mean absolute recovery of aciclovir and ganciclovir are 99.2 ± 2.5% and 100.3 ± 2.5% respectively. The method is precise (with mean inter-day CVs within 1.0-1.6% for aciclovir and 1.2-3.5% for ganciclovir), and accurate (range of inter-day deviations –1.6 to +1.6% for aciclovir and –0.4 to +1.4 for ganciclovir). The method has been applied in stability studies of ganciclovir in patients' blood samples, demonstrating its good stability in plasma at -20°C and at room temperature. The distribution of ganciclovir and aciclovir in plasma and red blood cells was also investigated *in vitro* in spiking experiments with whole blood, which showed an initial drop of ganciclovir and aciclovir levels in plasma (about -25%) due to the cellular uptake of aciclovir and ganciclovir by red blood cells. The method has been validated and is currently applied in a clinical study assessing the ganciclovir plasma concentration variability after administration of valganciclovir in a population of solid organ transplant patients.

3.2. Introduction

Cytomegalovirus (CMV) is the most important pathogen affecting transplant recipients [1]. CMV is known to cause both direct and indirect effects, including acute and chronic allograft rejection [2]. To prevent the burden of this infection in solid organ transplant (SOT) patients,

antiviral drugs are commonly used for both CMV prophylaxis and treatment. Intravenous administration of ganciclovir (figure 1), an acyclic guanosine analogue, has been the gold standard for the *treatment* of established infection, while valaciclovir (a prodrug of aciclovir, figure 1) and oral ganciclovir were administered for CMV *prophylaxis*. Valganciclovir, the valyl ester of ganciclovir, has been recently developed and is characterised by a near ten-fold higher bioavailability than ganciclovir. Valganciclovir thus offers the perspective of replacing suboptimal oral prophylactic (valaciclovir or oral ganciclovir) and intravenous therapeutic regimens.

Valganciclovir is hydrolysed in the intestinal wall and liver to L-valine and ganciclovir. Ganciclovir, after activation via triphosphorylation by virus and host cell enzymes, inhibits viral DNA polymerase and blocks viral DNA synthesis. The efficacy of valganciclovir has been formally validated by randomised controlled studies in the treatment of CMV retinitis in HIV patients and in the prophylaxis of CMV infection among high-risk (donor CMV seropositive/recipient CMV seronegative, D+/R-) kidney, liver and heart transplant recipients [3,4].

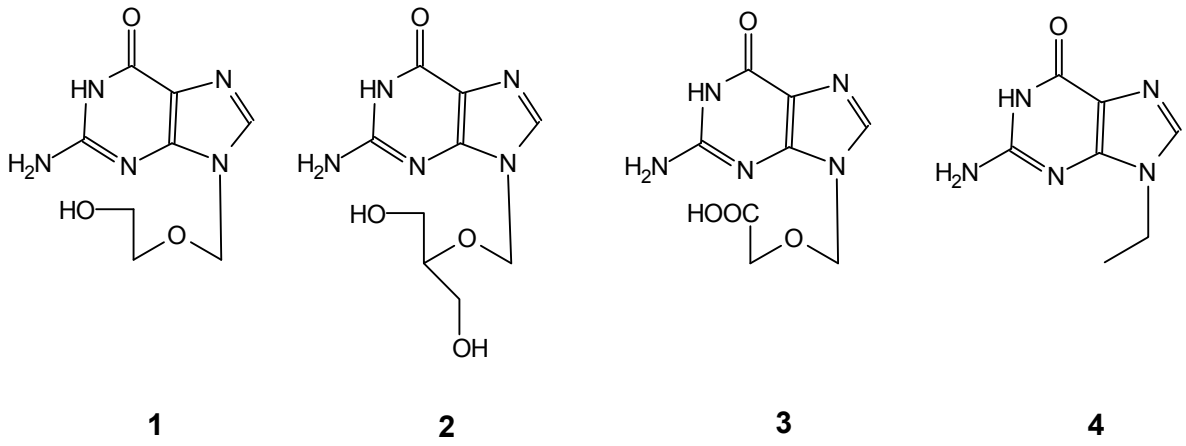

Figure 1: Aciclovir (1), ganciclovir (2), 9-carboxymethoxymethylguanine (CMMG) (3) and 9-ethyl-guanine (I.S.) (4) chemical structure

A number of pharmacokinetic studies of the parent drug ganciclovir are already available and suggest, according to Scott et al. [5], that the routine therapeutic drug monitoring of ganciclovir in SOT patients is of limited clinical usefulness. By contrast, little is known about the pharmacokinetics of ganciclovir after administration of its pro-drug valganciclovir in SOT patients. Pescovitz et al. have studied the pharmacokinetics over 24 hours of a single dose of oral valganciclovir in comparison to oral and intravenous ganciclovir in twenty-eight liver transplant recipients [6]. Whiltshire et al. have analysed the pharmacokinetic profile of ganciclovir after administration of valganciclovir in high-risk (D+/R-) kidney, liver and heart transplant recipients during prophylaxis regimens [7]. Yet, the usefulness of *routine clinical*

monitoring of ganciclovir plasma levels in SOT patients under prophylaxis or treatment with valganciclovir has never been evaluated. For example, ganciclovir is extensively eliminated by the kidney and patients with renal failure require dose adjustment. Ganciclovir is a substrate of renal tubular organic anion transporters, an active clearance system which is increasingly recognised as an important target in renal drug interactions [8]. In addition, there are no data on valganciclovir pharmacokinetics in transplant patients with cystic fibrosis, who are known to have gastrointestinal absorption problems and enhanced renal clearance, leading possibly to reduced systemic exposure to ganciclovir.

Nevertheless, a number of analytical methods have been proposed for the measurement of ganciclovir in biological fluids by high performance liquid chromatography (HPLC), but only a few enable the measurement of aciclovir and/or ganciclovir with the same assay, and none have considered the influence of the metabolite of aciclovir, 9-carboxymethoxymethylguanine (CMMG, figure 1) [9,10,11] that can potentially interfere with ganciclovir. In fact, CMMG, which has been already analyzed together with aciclovir by Svensson et al. [12], is at risk of co-eluting with ganciclovir when the latter is used as internal standard, or when previously or subsequently used in the same patient.

In addition, there remained some uncertainties on the stability of ganciclovir in blood samples. While most stability studies of ganciclovir have been performed in plasma - where it was generally found stable - one report raised the concern that ganciclovir may not be stable in whole blood left at room temperature [13], which resulted in stringent recommendations to store blood samples on ice immediately after blood sample collection prior to their transportation and centrifugation at low temperature. As ganciclovir is known to be subjected to active intra-erythrocyte uptake via transmembrane transport proteins [14], it was therefore necessary to ascertain whether those mechanisms would affect ganciclovir plasma levels during *in vitro* experiments, and *in vivo,* in patients blood samples left at room temperature.

We describe here a sensitive method for the assay of ganciclovir and aciclovir in human plasma, by HPLC coupled with spectrofluorimetric detection. This assay reaches the required level of sensitivity and reproductibility for routine clinical application and has also been applied in *in vitro* studies focusing on ganciclovir stability in blood samples.

This assay is currently applied in a research protocol aimed at assessing the interindividual and residual intraindividual variability of ganciclovir after administration of valganciclovir in solid organ transplant patients.

3.3. Materials and method

3.3.1. Chemicals

Zovirax™ vials (sodium aciclovir, corresponding to aciclovir 250 mg) and Cymevene™ vials (sodium ganciclovir, corresponding to ganciclovir 500 mg) were obtained from GlaxoSmithKline AG (Münchenbuchsee, Switzerland) and from Roche Pharma (Reinach, Switzerland), respectively. Aciclovir and ganciclovir stock solution (1 mg/ml) in ultrapure H_2O was prepared as follow: each extract was reconstituted with 10.0 ml H_2O in the vial, yielding a solution of aciclovir 25 mg/ml and ganciclovir 50 mg/ml: an aliquot of 2.0 ml of aciclovir 25 mg/ml and 1.0 ml of ganciclovir 50 mg/ml were subsequently diluted to 50.0 ml with ultrapure H_2O. Stock solution of 9-ethyl-guanine (Internal Standard, I.S., figure 1) at a concentration of 100 µg/ml was obtained by dissolution of 9-ethyl-guanine (Sigma, Switzerland) (3 min sonication) in 89 ml purified H_2O onto which 1.0 ml of acetic acid and 10.0 ml of methanol have been added. This solution was diluted down to 5 µg/ml before use with ultrapure H_2O. Acetonitrile (MeCN) for chromatography LiChrosolv®, 100% acetic acid (AcOH), trichloroacetic acid solution (TCA) 20% and heptane-1-sulfonic acid sodium salt LiChrosolv® ($C_7H_{15}NaO_3S$) were from E. Merck (Darmstadt, Germany). All other chemicals were of analytical grade and used as received. Ultrapure water was obtained from a Milli-Q® UF-Plus apparatus (Millipore).

3.3.2. Chromatographic system

The chromatographic system consisted of a Hewlett-Packard Series 1050 (Agilent, formerly Hewlett-Packard, Germany) pump equipped with an HP 1100 on line degasser, an HP 1050 autosampler and connected *via* an HP 35900 AC/DC interface to a spectrofluorimetric detector LC240 (Perkin-Elmer, Boston, USA) set at 260 nm (excitation) and 380 nm (emission). The separation was performed at room temperature (RT) on a ChromCart® cartridge column (250 x 4 mm I.D.) filled with Nucleosil 100-5 µm C18 (Macherey-Nagel, Düren, Germany) and equipped with a guard column (8 x 4 mm I.D.) filled with the same packing material. The injection volume was 30 µl.

The HP-ChemStation A.06.03 software was used to pilot the HPLC instrument and to process the data (area integration, calculation and plotting of chromatograms) throughout the method validation. Baselines were visually inspected and were manually adjusted (in general, base line to base line) using peak start and end features of the HP-ChemStation software.

3.3.3. Mobile phase solutions

Solution A was prepared, prior to each series of analysis, by dissolution of 4.0 g sodium heptanosulfonate with ultrapure H_2O up to 1000.0 ml with pH carefully adjusted to 2.60 with 100% acetic acid. Solvent B consisted of pure MeCN. The mobile phase was delivered at 1 ml/min and the gradient elution program was: solvent A: 100% at 0.00 min→ 93% at 19.00 min→ 86% at 31.00 min→ 0% at 31.01 min→ 0% at 36.00 min→ 100% at 36.01→ 100% at 42.00 min.

3.3.4. Stock solution, working solution, plasma calibration and control samples

Stock solution of aciclovir and ganciclovir at 1 mg/ml in H_2O was further diluted with H_2O for the preparation of working solutions at concentrations of 1-100 µg/ml. Plasma calibration samples at 0.1, 0.25, 0.50, 1.0, 5.0, 10.0 µg/ml, together with plasma quality control samples at 0.75, 3.0, 8.0 µg/ml, were prepared by 1 :10 dilution of the respective working solution with blank plasma from outdated transfusion bags (total added volume $\leq$ 10% of the biological sample volume), in accordance with the recommendations on bioanalytical method validation [15,16].

The calibration standards and control samples were prepared in batches at the same occasion and were stored at -20°C as 1.2 ml aliquots in 5 ml-polypropylene Eppendorf tubes, and thawed on the day of analysis.

3.3.5. Samples collection

According to a study protocol previously approved by the Ethics Committee of University Hospital, blood samples were taken from solid organ transplant patients under prophylaxis or treatment with valganciclovir during their hospital stay and for their subsequent routine follow-up at their scheduled medical visits. Blood samples (5.5 ml) were collected in Monovettes® (Sarstedt, Nümbrecht, Germany), with K-EDTA as anticoagulant. Samples were sent without delay to the laboratory and were centrifuged at 1850 g (3000 rpm) for 10 min at 4°C (Beckmann Centrifuge, Model J6B) and the plasma was separated and transferred into 5 ml-polypropylene test tubes before being stored at –20°C up to the time of analysis.

3.3.6. Sample preparation

On the day of analysis, calibration, quality control and patient samples were thawed, allowed to equilibrate at RT and vortex-mixed. Aliquots (1000 µl) of plasma samples (calibration,

control, patients) with 250 μl of I.S. solution (5 μg/ml) was vortexed in an Eppendorf vial before protein precipitation with 250 μl of TCA 20%. After being vortex-mixed the suspensions were centrifuged for 10.0 min on a benchtop centrifuge at 20'000 g (14'000 rpm) at 4°C (Hettich® Benchtop Universal 16R centrifuge, Bäch, Switzerland). The supernatants were collected and evaporated to dryness under a nitrogen steam at 37°C for approximately 2.5 hours. The residues were then reconstituted in 100 µl of mobile phase solution A. The resulting solutions were carefully vortexed twice, transferred to Eppendorf microvials and then centrifuged at 20'000 g for 10 min at 4°C. The supernatents were introduced into 0.5 ml HPLC autosampler vials (Laubscher Labs, Switzerland) and a volume of 30 µl was used for HPLC analysis.

3.3.7. Calibration curves

Quantitative analysis of aciclovir and ganciclovir was performed using the Internal Standard (I.S. = 9-ethyl-guanine) method.

The calibration curves were fitted by least-squares linear regression using 1/concentration (1/x) as weighting factor of the peak-area ratio of aciclovir and ganciclovir to I.S. versus the ratio of the injected amount of the respective aciclovir and ganciclovir to I.S., in each standard samples. The calibration was established over the clinically relevant range 0.1-10.0 µg/ml for aciclovir and ganciclovir.

9-ethyl-guanine was chosen as internal standard, because this synthetic guanine derivative is a structural analogue of aciclovir and ganciclovir, and is unlikely to be present in patient samples.

3.3.8. Analytical method validation

The validation of the method was based on the guidelines published on-line by the FDA [17] as well as on the recommendations of the Conference Report of the Washington Conference on "Analytical methods validation: Bioavailability, Bioequivalence and Pharmacokinetic studies" [15] and of the Arlington Workshop "Bioanalytical Methods Validation – A revisit with a Decade of Progress" [16].

Each level of the calibration curve was established after two injections of each calibration samples: one at the beginning and the second at the end of the run. Throughout patient sample analysis, control samples at three concentrations levels (low, medium and high: i.e. 0.75, 3.0 and 8.0 µg/ml) were assayed at least every five samples.

Replicate analysis (n=6) of quality control samples were used for the determination of the precision and accuracy of the assay, the three concentrations were chosen to encompass the range of the calibration curve corresponding to aciclovir and ganciclovir levels expected to occur in patient samples. Precision being calculated as the coefficient of variation (C.V.%) within a single run (intra-assay) and between different assays (inter-assay), and the accuracy as the percentage of deviation between nominal and measured concentration.
Both experimental lower limit of quantification (LLOQ) and limit of detection (LOD) were determined by diluting the calibration samples. The LLOQ for aciclovir and ganciclovir in plasma was experimentally chosen as the minimal concentration in plasma samples which could be confidently determined in accordance with the Conference Report on Analytical method validation [15,16] and the FDA [17] recommending that the deviation between measured and nominal concentration at LLOQ should not deviate more than ± 20%. The limit of detection (LOD) was considered as the concentration of aciclovir and ganciclovir that provides a signal corresponding to 3 times the HPLC background signal.

3.3.9. Recovery

The efficiency of the sample preparation by protein precipitation with TCA 20% was determined with quality control samples at three levels (0.75, 3.0 and 8.0 µg/ml of aciclovir and ganciclovir, n = 3 for each level). The absolute recovery of aciclovir and ganciclovir from plasma was obtained as the peak-area response of the processed sample, expressed as a percentage of the response of the same amount of aciclovir and ganciclovir, calculated to be contained into the 30 µl- injection volume reconstituted in solution A, which corresponds to the 100% recovery.

3.3.10. Stability of aciclovir and ganciclovir

Stability studies of aciclovir and ganciclovir included:
1. Long term stability of plasma samples kept frozen at –20°C: six series of calibration and quality control plasma samples spiked with aciclovir and ganciclovir were prepared. Three series were either immediately analysed (i.e. without being frozen) while the three remaining series were stored during 4 months at –20°C. The slope of the calibrations curves were compared (Student's *t*-test).
2. Samples stability at 4°C and at room temperature
    a) Stability of aciclovir and ganciclovir in *plasma* samples at 4°C and at room temperature: six series of calibration and quality control plasma samples spiked with

aciclovir and ganciclovir were prepared. Two series were immediately frozen at –20°C. Two series were kept at RT for 24 hours before being frozen at –20°C. Two series were stored for 24 hours at 4°C and then frozen at –20°C. The slopes of aciclovir and ganciclovir calibration curves in both groups were compared (Student's *t*-test).

b) Stability of aciclovir and ganciclovir in *blood* samples at 4°C and at room temperature: six series of calibration and quality control citrated blood samples spiked with aciclovir and ganciclovir in 0.9% NaCl were prepared. Two series were immediately centrifuged at 1850 g (3000 rpm) for 10 min at +4°C. The plasma was collected and frozen at –20°C. Two other series of blood samples were kept at RT for 48 hours, then centrifuged and frozen at –20°C. Two series of blood were stored for 48 hours at 4°C before being centrifuged and frozen at –20°C. The slopes of aciclovir and ganciclovir calibration curves in both groups were compared (Student's *t*-test).

c) Kinetics (concentration *vs* time profile) of the distribution of aciclovir and ganciclovir in plasma and red blood cells from *blood* samples left at room temperature were also assessed: A 100 ml citrated blood sample spiked with aciclovir and ganciclovir at 7000 ng/ml in 0.9% NaCl were prepared. At 0, 1, 2, 3, 4, 6, 8, 24, 30 and 48 h, two samples (3 ml) were centrifuged at 1850 g (3000 rpm) for 10 min at 4°C. Plasma and remaining cellular components were separated and frozen at –20°C prior to analysis. For the determination of aciclovir and ganciclovir in red blood cells (RBC), haemolysed cellular samples were analysed using calibration and quality control prepared with haemolysed RBC pellets samples instead of plasma.

d) Stability of ganciclovir in *patients' blood* samples: six blood samples collected from patients at the occasion of their medical visits were divided in two aliquots. One blood aliquot was immediately centrifuged at 1850 g (3000 rpm) for 10 min at 4°C and the plasma was collected and frozen at –20°C. The other blood aliquot was kept at RT for 24 hours (in 3 patients) or 48 hours (in 3 other patients) prior to centrifugation and plasma storage at –20°C. The variations of ganciclovir plasma concentrations over time were expressed as a percentage of the levels determined in samples immediately centrifuged.

3. Stability of plasma samples after multiple freeze-thaw cycles: aliquots of plasma spiked with aciclovir and ganciclovir at 0.75, 3.0 and 8.0 µg/ml, underwent three freeze-thaw cycles: frozen samples were allowed to thaw at ambient temperature for 2 hours and were subsequently refrozen. Aciclovir and ganciclovir levels were measured in aliquots from the three consecutive freeze-thaw cycles and were analysed in the same series, to eliminate the

inter-assay variability. The variations of aciclovir and ganciclovir concentrations were expressed in percentage of the levels of samples not subjected to the freeze-thaw cycles.

4. Stability of plasma extracts into HPLC vials at room temperature: processed calibration and quality control samples spiked with aciclovir and ganciclovir (i.e. reconstituted in solution A) were analysed in duplicate either immediately after preparation, or after being left 24 h and 48 h at room temperature in the auto-sampler rack. The variations of aciclovir and ganciclovir concentrations were expressed in mean percentage of change of the initial concentration.

5. In case some samples would require HIV viro-inactivation, the stability of aciclovir and ganciclovir in plasma under the recommended thermisation process (60°C for 60 min) [18-21] was assessed as follows: four series of calibration samples at the six concentrations reported above (0.1 up to 10.0 µg/ml) were analysed in parallel. Two were heated at 60°C for 60 min, while the thermisation procedure was omitted for the two other series. The slope was compared between the resulting calibration curves (Student's *t*-test).

3.3.11. Selectivity

The selectivity of our analytical method was determined by injecting onto the HPLC column blank plasma from 25 different subjects and the following drugs: acenocoumarol, acetaminophen, acetylcysteine, acetylsalicylic acid, allopurinol, amlodipin, amoxicillin, amprenavir, atazanavir, atenolol, atorvastatin, azathioprin, azithromycin, bromazepam, cafein, candesartan, ciclosporin, cefepim, ceftazidim, chlortalidone, cilastatin, ciprofloxacin, cisaprid, clavulanic acid, codein, diazepam, diclofenac, efavirenz, enalapril, fludrocortisone, fluconazol, folic acid, furosemide, guaifenesin, ibuprofen, imipenem, indinavir, lamivudin, levofloxacin, loperamid, lopinavir, lorazepam, mefenamic acid, meropenem, metamizol, metoprolol, mycophenolate mofetyl, nelfinavir, nevirapine, omeprazol, oxazepam, phytomenadion, piperacillin, prednison, risedronat, spironolactone, sulfamethoxazol, rifampicine, ritonavir, saquinavir, sulfasalazine, tacrolimus, tazobactam, tipranavir, torasemid, tramadol, trimethoprim, uric acid, vancomycin, zidovudin.

3.3.12. Applications of the HPLC method

This method is currently applied in a research protocol, approved by the local Ethics Committee, aimed at assessing the interindividual and residual intraindividual variability of ganciclovir after administration of valganciclovir in solid organ transplant patients who require prophylaxis or treatment of cytomegalovirus infection, using a population pharmacokinetic

approach, and at determining the relation between ganciclovir plasma concentration and the virological (viremic charge) and clinical (CMV disease) outcomes.

3.4. Results and discussion

3.4.1. Chromatograms

The proposed HPLC method enables the measurement of aciclovir and ganciclovir in plasma with fluorimetric detection at 260 nm (emission) and 380 nm (detection). With the gradient program used, the retention times for ganciclovir, aciclovir and 9-ethyl-guanine are 13 min, 15 min, and 30 min, respectively. This gradient elution program yields sharp peaks without producing any significant drift of the baseline. The entire HPLC run (including the rinsing and re-equilibration step) lasts 42 min to achieve an excellent separation of aciclovir and ganciclovir from endogenous plasma components with satisfactory selectivity towards the aciclovir metabolite CMMG (see below).

The chromatograms of a blank plasma and of the quality control at 3.0 µg/ml of aciclovir and ganciclovir (onto which the 9-ethyl-guanine (I.S.) has been added) are shown in figures 2 and 3, respectively. The potential interference between ganciclovir and the aciclovir metabolite CMMG (figure 1) has also been studied. Using the elution conditions proposed with the solution A in the mobile phase adjusted to pH 2.60, the separation of CMMG and ganciclovir was not found optimal. However, a minor pH modification of solution A to pH 2.90 enabled, with a slight change of their retention time, a satisfactory resolution of CMMG and ganciclovir (difference in retention time of $\approx$ 0.6 min). Figure 4 shows the chromatogram of a plasma quality control sample of aciclovir, ganciclovir (750 ng/ml) spiked with the aciclovir metabolite CMMG at a concentration of 1500 ng/ml using the proposed gradient elution program at pH 2.90. Thus, in the rare clinical instances where aciclovir and ganciclovir would be administered to a same patient (i.e. during treatment switch), CMMG and ganciclovir can be nevertheless efficiently separated by a slight pH modification. More generally however, caution should be exercised if ganciclovir is considered as internal standard for aciclovir determination [10,22,23].

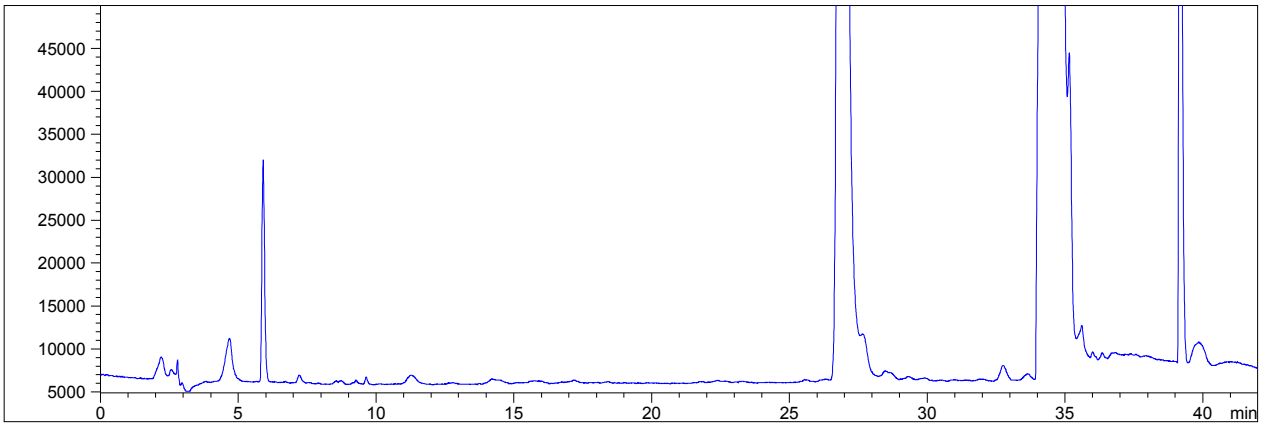

Figure 2: Chromatographic profile of a blank plasma

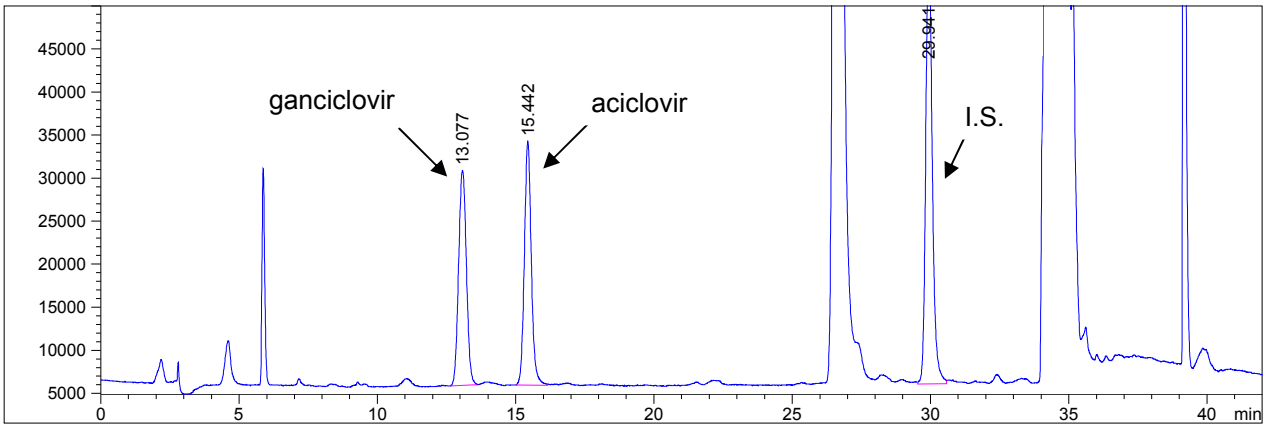

Figure 3: Chromatographic profile of a plasma sample of aciclovir and ganciclovir (3000 ng/ml) spiked with I.S. (9-ethyl-guanine)

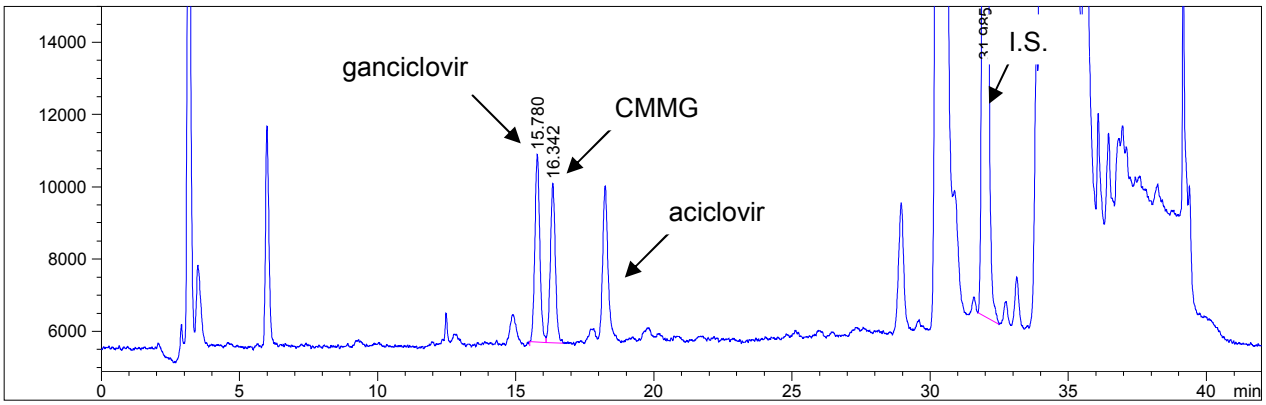

Figure 4: Chromatographic profile of a plasma quality control sample (20 µl) of aciclovir, ganciclovir (750 ng/ml) spiked with 10 µl of CMMG (1500 ng/ml) and with I.S. (9-ethyl-guanine)

3.4.2. Mobile phase composition

For prolonged routine analyses, it is particularly important to control the elution conditions, especially the pH of solution A (at 2.60) and gradient program, to ensure consistent peak shape and retention time of aciclovir and ganciclovir and for satisfactory separation from matrix peaks. Solvent was found stable at room temperature up to 48 hours: the pH remained identical (pH ± 0.04) during this period of time and there were no signs of microbiological contamination.

3.4.3. Calibration curves

The calibrations curves have been calculated and fitted by least-squares linear regression either unweighted, or using 1/concentration (1/x), and 1/concentration^2 (1/x^2) as weighting factor. To establish the best weighting factor, back-calculated concentrations were determined. The model with the lowest total bias and the most constant bias across the concentration range was considered to be the best fit. Visual inspection of the plot of residuals of the 1/x weighted regression indicates that there are no trend in variability throughout the delineated range of concentrations. Moreover, the homogeneity of variances of the residuals have been statistically verified according to Levene's test [24] yielding Pr values > F = 0.08 for aciclovir and > F = 0.4 for ganciclovir, verifying the hypothesis of homoscedasticity, and indicating that the chosen 1/x model was indeed adequate.

The slope of the calibration curves appeared stable, with values averaging 0.89 (± 4.6%) (n=6) and 0.90 (± 1.7%) (n=6) for ganciclovir and aciclovir, respectively. Over the concentration range 0.1-10.0 µg/ml for aciclovir and ganciclovir, the regression coefficient r^2 of the calibration curves remained excellent, always greater than 0.999.

The calibration samples are prepared with citrate plasma whereas patients' plasma samples are collected on EDTA Monovettes®. For the sake of validation, a cross-comparison has been performed between three series of three levels of QC determined against calibration curves established on both matrices (citrate *versus* EDTA). The results of the head-to-head comparison reveal a small albeit significant difference between both anticoagulants ($P < 0.05$, two-way ANOVA), with a mean relative differences of 0.7% ± 3.2%, and of 2.5% ± 3.4%, for ganciclovir and aciclovir respectively, if EDTA samples are read against citrate curve. Considering these values and the intra- and inter-assay accuracy (bias) (table 1), no correction of the results deserves to be applied when the analysis of EDTA samples rather than citrate samples is performed against citrate calibrators.

3.4.4. Validation of the HPLC method: precision, accuracy and LLOQ/LOD

Precision and accuracy achieved with control samples are given in table 1. The concentration levels of control samples of aciclovir and ganciclovir (750, 3000 and 8000 ng/ml) were selected to encompass the clinically relevant range of concentrations expected in plasma samples.

Throughout these concentration ranges, the mean intra-assay precision was similar, always lower than 2%. Overall, the mean inter-day precision for aciclovir and ganciclovir was good with mean CVs within 1.0 – 1.6% and 1.2 – 3.5%, respectively. The intra-assay deviation (bias) from the nominal concentrations of aciclovir and of ganciclovir was comprised between –0.4 to +0.8% and -0.4 to +1.4%, respectively, and the range of inter-day deviation was always < 1.6% and < -1.4%, respectively.

Table 1: Precision and accuracy of the assay for aciclovir and ganciclovir in plasma (750, 3000 and 8000 ng/ml)

Nominal concentration (ng/ml)	Concentration found (ng/ml)	SD (±)	Precision CV (%)	Accuracy* bias (%)
ACICLOVIR				
A. Intra-assay (*n*=6)				
750	747	1	0.2	-0.4
3000	3024	52	1.7	0.8
8000	7979	63	0.8	-0.3
B. Inter-assay (*n*=6)				
750	762	13	1.6	1.6
3000	2995	45	1.5	-0.2
8000	7869	80	1.0	-1.6
GANCICLOVIR				
A. Intra-assay (*n*=6)				
750	747	4	0.6	-0.4
3000	3042	49	1.6	1.4
8000	7984	53	0.7	-0.2
B. Inter-assay (*n*=6)				
750	746	25	3.5	-0.4
3000	2957	48	1.6	-1.4
8000	7885	91	1.2	-1.4

*(Found—nominal)/nominal x 100.

By analysing plasma from outdated transfusion bags spiked with decreasing concentrations of aciclovir and ganciclovir (50-12.5 ng/ml), the limit of detection (LOD) was experimentally found to be 25 ng/ml. The lower limit of quantification (LLOQ) of aciclovir and ganciclovir, independently determined by back-calculation, is 100 ng/ml. The precision (C.V.) is 4.2% and 5.8% respectively and the accuracy (i.e. bias, calculated by back-calculation) at this level is – 1.6% and - 2.1% respectively. As both values are thus comprised well within the ± 20% limit recommended by the Arlington Workshop [16], 100 ng/ml was therefore chosen as the lower level of calibration.

3.4.5. Recovery

The mean absolute recovery of aciclovir and ganciclovir measured with the high, medium and low QC controls were 99.2 ± 2.5% and 100.3 ± 2.5% respectively. The protein precipitation with TCA 20% was found to be a reliable way of eliminating plasma protein with a high absolute recovery and low recovery variability. The internal standard is fully recovered at the concentration spiked (5000 ng/ml) with a low variability: 98.6 ± 0.1%.

3.4.6. Samples stability

Stability of plasma samples at -20°C

No evidence of aciclovir and ganciclovir decomposition was found during plasma samples storage in the freezer at -20°C for at least 4 months. In fact, for aciclovir and ganciclovir, the mean slope of calibration curves (n=3) established with samples let 4 months at -20°C (m=0.81 and 0.85, respectively) was not different than the slope of calibration curves calculated with samples analysed immediately (m=0.80 and 0.85; n=3), (P = 0.2 and 0.5, Student's *t*-test) indicating good aciclovir and ganciclovir stability in plasma at -20°C for at least 4 months.

Stability at 4°C and at room temperature

The stability of *plasma* samples left at 4°C and at room temperature was ascertained up to 24 h. For aciclovir, the mean slope of calibration curves (n=2) established with plasma samples left 24 hours at 4°C and at RT (m=0.84 and 0.83, respectively) was not different than the slope of calibration curves calculated with samples stored during the same time at – 20°C (m=0.80; n=2), (P = 0.1 and 0.3, at 4°C and at RT, respectively; Student's *t*-test), indicating good stability of aciclovir in plasma at RT. For ganciclovir, the mean slope of

calibration curves (n=2) established with samples left 24 hours at 4°C and at RT (m=0.84 and 0.83 respectively) did not differ from the slope of calibration curves calculated with samples stored during the same time at –20°C (m=0.82; n=2), (P = 0.2 and 0.3, at 4°C and at RT, respectively; Student's *t*-test), also indicating good stability of ganciclovir in plasma at RT.

The stability of aciclovir and ganciclovir spiked to *blood* samples left at room temperature and at 4°C was also checked. After 48 h, there was a significant decrease in plasma levels collected from blood left both at RT and at 4°C (-17% and -15%, -28% and -24%, for aciclovir and ganciclovir, respectively, $P < 0.05$, Student's *t*-test) in comparison to plasma collected immediately after the addition of aciclovir and ganciclovir to blood. A substantial decrease in aciclovir and ganciclovir plasma concentrations observed *in vitro* after the addition of aciclovir and ganciclovir into blood has been previously reported [13] and is most probably due to the cellular uptake by erythrocytes *via* purine nucleobase carriers and nucleoside transporters, of which aciclovir and ganciclovir are known to be substrates [14]. The distribution of aciclovir and ganciclovir in plasma and in red blood cells *in vitro* after their addition into *whole blood* left at room temperature was therefore studied in more details. Figure 5 and 6 show that there is a pronounced initial drop in aciclovir and ganciclovir concentration in plasma, –26% and – 22% after 1 hour, followed by a less marked decrease, – 32% and – 35% after 48 hours, for aciclovir and ganciclovir, respectively. This is accompanied by a corresponding increase in aciclovir and ganciclovir levels in haemolysed erythrocytes, supporting the hypothesis that the observed decrease in aciclovir and ganciclovir concentrations in plasma *in vitro* is indeed due to drug uptake by erythrocytes until erythrocyte/plasma equilibrium is reached (in our case, the erythrocyte/plasma ratio was 1.11 ± 0.06). *In vivo* however, this phenomenon is unlikely to affect the accuracy of drug measurements in blood from patients treated for a few day with ganciclovir or valganciclovir, as the drug distribution in cell and plasma has already reached an equilibrium in circulating blood at the time of sampling. Indeed, the stability of ganciclovir in plasma was assessed in three patients whose anticoagulated blood samples were left at room temperature for 24h or for 48h, showing a bias of -1.5% and +3.5% respectively, demonstrating the good stability of ganciclovir in plasma from blood samples left at least for 48h at RT after collection from patients. This is of particular interest for samples shipment in the perspective of multicentric studies. Our observations contrast to those of Boulieu et al. [13] who have previously found a limited stability of ganciclovir, necessitating, according to these authors, stringent conditions of samples collection.

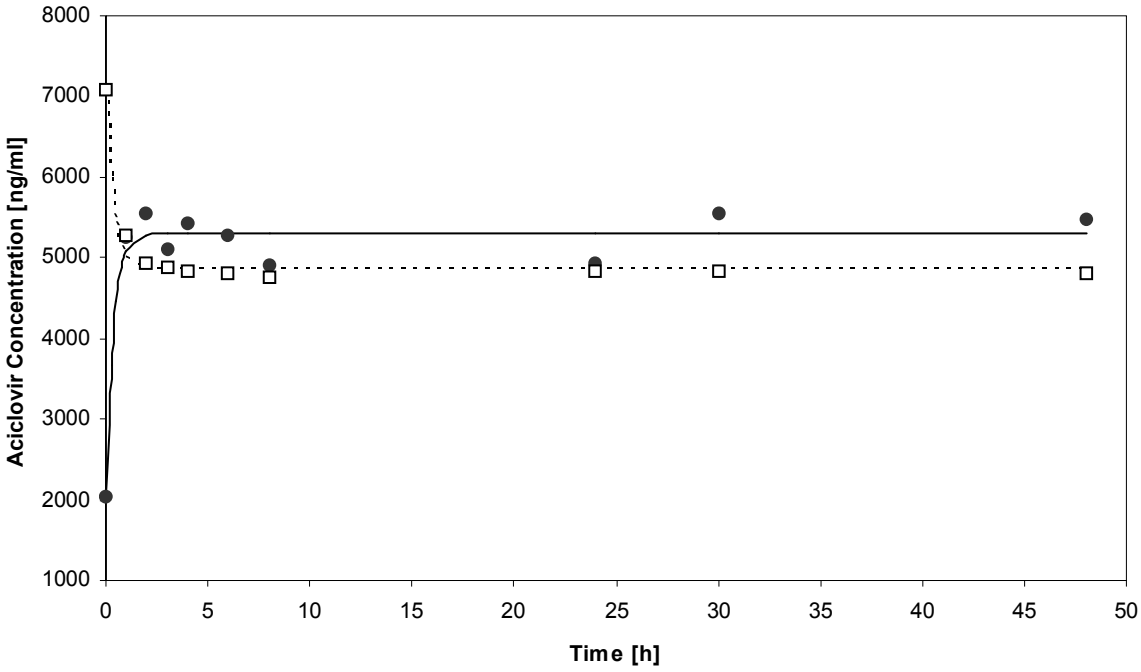

Figure 5: Aciclovir concentrations profile after the addition of aciclovir at 7000 ng/ml (in 0.9% NaCl) in whole anticoagulated blood. Concentration in plasma (□) and in haemolysed erythrocyte (●). The equilibrium is reached according to an exponential curve, fitted by least-square regression.

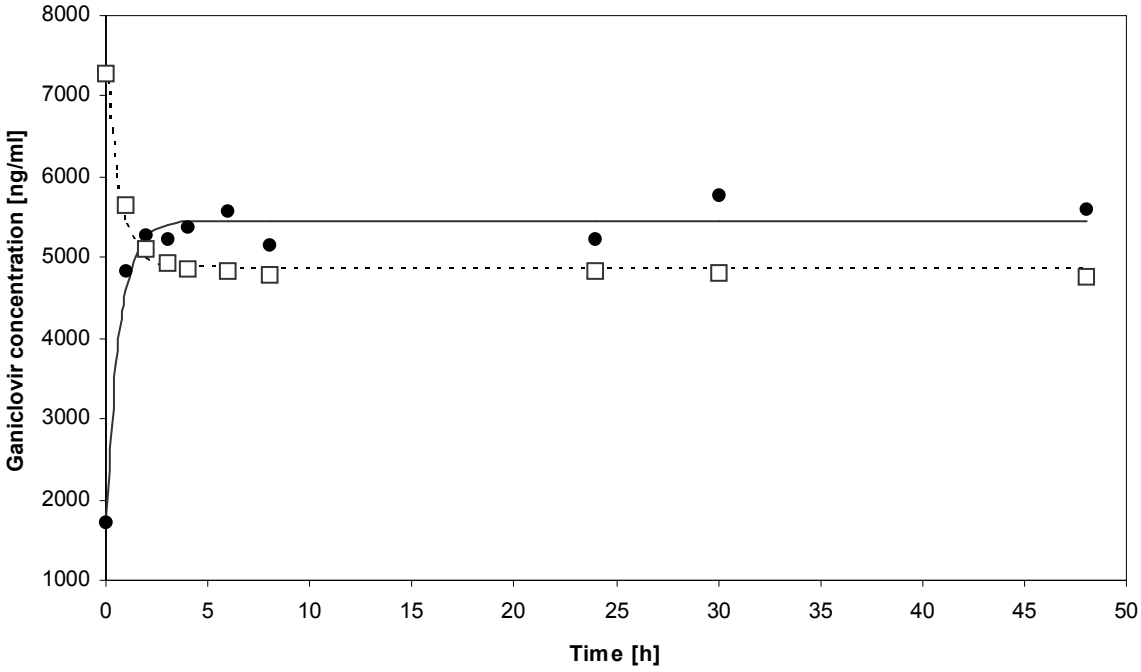

Figure 6: Ganciclovir concentrations profile after the addition of ganciclovir at 7000 ng/ml in 0.9% NaCl) in whole anticoagulated blood. Concentration in plasma (□) and in haemolysed erythrocyte (●). The equilibrium is reached according to an exponential curve, fitted by least-square regression.

Stability of plasma samples after one, two and three freeze-thaw cycles

The variations of aciclovir and ganciclovir concentrations when submitting control plasma to three successive freeze-thaw cycles are reported in table 2. This indicates that no significant loss of aciclovir and ganciclovir is to be expected after up to three freeze-thaw cycles.

Table 2: Stability of aciclovir and ganciclovir plasma samples after one, two and three freeze-thaw cycles for the QC samples at nominal concentration of 750, 3000, 8000 ng/ml, respectively. (concentration change expressed in % of the initial concentration)

n° of thaw-freeze cycles	ACICLOVIR Nominal Concentration			GANCICLOVIR Nominal Concentration		
	750	3000	8000	750	3000	8000
1	97.9 ± 1.3	96.3 ± 1.0	99.0 ± 0.3	99.4 ± 0.8	100.3 ± 0.3	100.3 ± 0.4
2	93.9 ± 2.5	97.6 ± 0.2	105.6 ± 2.1	95.5 ± 2.2	97.4 ± 0.5	99.0 ± 1.0
3	100.2 ± 0.2	102.6 ± 1.9	105.7 ± 0.8	98.0 ± 0.9	97.8 ± 0.1	97.8 ± 0.4

Stability of extracts samples into HPLC vials (i.e. ready for HPLC analysis) at room temperature

The stability of plasma extracts (i.e. reconstituted in solution A, in HPLC vials) submitted to HPLC analysis was checked at RT for 24 and 48h and is reported in table 3. The variations of drug concentration, for aciclovir and ganciclovir respectively, over time in samples left at RT, expressed in percentage of the starting levels (i.e. after immediate analysis), were less than -8.1 ± 2.8% and -2.6 ± 1.6% after 24h, and less than –11.4 ± 0.8 and –9.1 ± 2.2 after 48h. This indicates that even though there is a slight decrease in aciclovir concentration in plasma extracts left after 24h at room temperature this difference is comprised within 10%, which is still acceptable if the HPLC run does not exceed one day. However, the extracted plasma in HPLC vials should not be left more than 24h at room temperature because of the apparent reduced stability after 48 h in this condition.

Table 3: Stability of aciclovir and ganciclovir in extract samples left at room temperature (RT) for 24 and 48h

Duration (hours)	Nominal Concentration (ng/ml)		
	750	3000	8000
ACICLOVIR*			
24	- 8.1 ± 2.8	- 6.8 ± 0.3	- 5.1 ± 0.4
48	- 11.4 ± 0.8	- 9.0 ± 0.4	- 9.5 ± 0.1
GANCICLOVIR*			
24	- 2.6 ±1.6	- 1.4 ± 1.3	- 1.1 ± 0.3
48	- 9.1 ± 2.2	- 8.9 ± 0.9	- 8.6 ± 0.9

* mean percentage change of the initial concentration ± SD; n = 2

Stability during thermisation (HIV inactivation)

The slope of the calibration curves of aciclovir and ganciclovir established in samples submitted to the thermisation procedure (60°C for 60 min) was slightly lower (variation of -6.2 ± 0.2%) than that obtained with non-heated samples for aciclovir, and not different for ganciclovir (0.5 ± 0.5%), as shown in table 4. This difference was however not significant for both compounds (P = 0.2 and 0.5, for aciclovir and ganciclovir respectively, Student's *t*-test), indicating that such a procedure does not affect to a significant extent aciclovir and ganciclovir concentrations, within the considered concentrations range. Thus, thermisation can be considered in case of HIV inactivation of samples is required.

Table 4: Parameters of the calibration curves for aciclovir and ganciclovir before and after plasma thermisation at 60°C for 60 min (n=2)

Sample Treatment (n=2)	*m*	r^2	*b*	Variation (%)
ACICLOVIR				
1/Thermisation 60 min at 60°C	0.8616	0.99984	1.073E-2	
1/No thermisation	0.9200	0.99980	1.087E-2	-6.4
2/Thermisation 60 min at 60°C	0.7903	0.99997	1.073E-2	
2/No thermisation	0.8409	0.99994	1.089E-2	-6.0
Mean ± S.D.				**-6.2 ± 0.2**
GANCICLOVIR				
1/Thermisation 60 min at 60°C	0.8625	0.99997	0.588E-3	
1/No thermisation	0.8704	0.99999	1.508E-3	0.9
2/Thermisation 60 min at 60°C	0.8102	0.99960	1.546E-3	
2/No thermisation	0.8114	0.99992	1.714e-3	0.1
Mean ± S.D.				**0.5 ± 0.5**

m: slope, r^2:coefficient of determination, *b*: y-axis intercept

3.4.7. Selectivity

Among the 25 different blank plasma tested, none showed the presence of significant interfering endogenous peaks at the retention time of aciclovir, ganciclovir and the internal standard. The method selectivity was confirmed by analysing the various immunosuppressive treatment regimens and more than 40 different other drugs possibly prescribed to transplant patients at our hospital, and 10 anti-HIV drugs. Few drugs were detected by spectofluorimetry at 380 nm, and all were eluted at times not perturbing aciclovir and ganciclovir analysis.

3.4.8. Clinical applications

This HPLC assay is currently applied to the analysis of samples collected as part of an ongoing clinical research study on the pharmacokinetics and pharmacodynamics of ganciclovir after administration of valganciclovir in solid organ transplant patients. For example, figure 7 shows the chromatographic profile of one plasma obtained from a SOT patient receiving prednisone, tacrolimus, and a prophylactic regimen of valganciclovir 450 mg once a day. The plasma level of ganciclovir measured 2 h 30 min after the Valcyte® intake is 3.1 µg/ml ($IC_{95\%}$ = 3.0-3.2 µg/ml).

Of note, ganciclovir and aciclovir drug levels measurements were in some instances asked for neonates and for pediatric patients for whom the volume of blood collected must be limited. In these cases, aliquots as low as 100 µl of plasma have been successfully analysed using the proposed method, using accordingly corresponding volumes of calibration and QC samples, with satisfactory quantification limits.

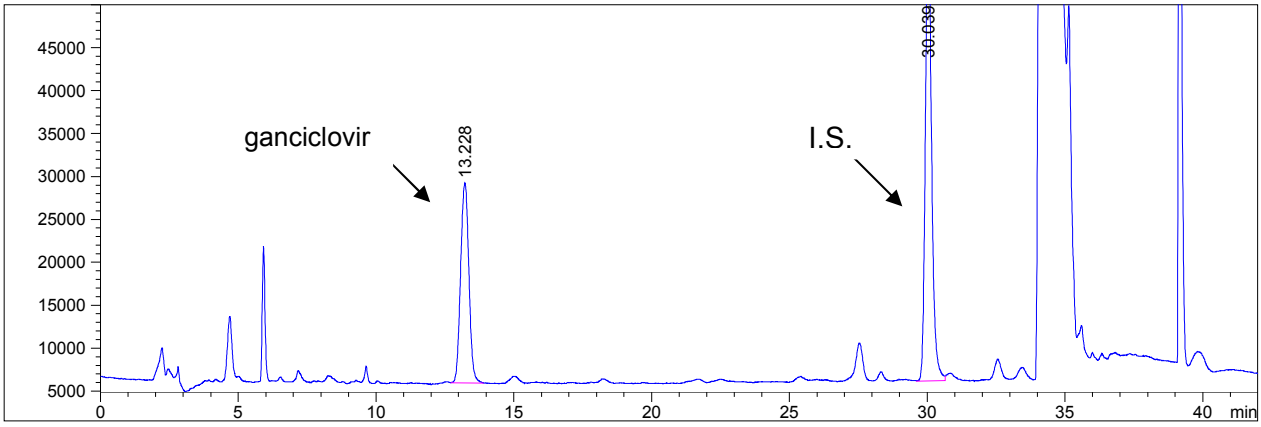

Figure 7: Chromatographic profile of a plasma from a solid organ transplant patient receiving valganciclovir

3.5. Conclusion

This HPLC method provides a simple and robust procedure for determining aciclovir or ganciclovir in patients' plasma. It has been developed using instruments available in conventional hospital laboratories, including a spectrofluorimetric detector. This procedure, through relatively time consuming, represents a practicable, cheap and robust method providing the required level of sensitivity for measuring clinically relevant ranges of concentrations of aciclovir and ganciclovir.

During this study, we have pointed out the potential interference of the metabolite of aciclovir, 9-carboxymethoxymethylguanine (CMMG) with ganciclovir, a problem that has been up to now only limitedly addressed. This interference potential does not represent a strong limitation of our analytical method since it can be easily circumvented by a slight pH change of the aqueous mobile phase to pH in the exceptional cases (i.e. during treatment switch) where ganciclovir and aciclovir would be present simultaneously in a same patient.
In addition, we have demonstrated the good stability of ganciclovir in patients' blood samples at room temperature up to 48h. Thus, according to our observations, patients' samples do not require the stringent sample collection conditions recommended by Boulieu et al. [13] Finally, since plasma extract samples are stable at room temperature in the autosampler rack over 24 hours, the duration of one analytical run does not represent a limitation of our method. Using a devoted HPLC apparatus, it is possible to analyze 20 patients' samples per analytical series. The method is currently applied for the monitoring of ganciclovir and aciclovir in SOT patients.

3.6. References

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Chapter 4 : Population pharmacokinetic study

Presentation of chapter 4

What is the average pharmacokinetic profile of valganciclovir in our population of patients? Which clinical factors influence it? Is the remaining variability important? Does it have an impact on treatment efficacy or toxicity? Should we advise the prescribers to monitor plasma routinely?

The following chapter represents the main part of this research, i.e. the clinical study itself [ISRCTN06404801]. This study was performed according to the good clinical practice (GCP).

First, the protocol and case report form (CRF) were developed and submitted to the local ethics committee and notified to Swissmedic. A database was developed to record all data, with the assistance of an informatician. Blood samples and clinical data (timing of last dose intake, sample collection time and current dose regimen, ..) were thoroughly collected. Ganciclovir plasma concentration was measured and the results were communicated to physicians. All data were analyzed using a population approach, performed with the dedicated programme Nonmem (non-linear mixed effect modelling). The elaboration of the final model is a demanding work, which required not less than 300 successive Nonmem runs and the supervision by a qualified expert.

Population pharmacokinetics of ganciclovir in solid organ transplant recipients receiving oral valganciclovir

N. Perrottet[1], C. Csajka[1], M. Pascual[2], O. Manuel[2], F. Lamoth[2], P. Meylan[3], J.D. Aubert[2,4], J.P. Venetz, P. Soccal[5], L.A. Decosterd[1], J. Biollaz[1], T. Buclin[1]

[1] Division of Clinical Pharmacology and Toxicology, University Hospital (CHUV), Lausanne, Switzerland

[2] Transplantation Centre, University Hospital (CHUV), Lausanne, Switzerland

[3] Microbiology Institute, University Hospital (CHUV), Lausanne, Switzerland

[4] Pneumology service, University Hospital (CHUV), Lausanne, Switzerland

[5] Pneumology service, University Hospital (HUG), Geneva, Switzerland

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Related appendices: 2.1, 2.2, 2.3, 2.4 and 2.5

4. Population pharmacokinetics of ganciclovir in solid organ transplant recipients receiving oral valganciclovir

4.1. Abstract

Valganciclovir (VGC) is an oral prodrug of ganciclovir (GCV) recently introduced for prophylaxis and treatment of cytomegalovirus infection. Optimal concentration exposure for effective and safe VGC therapy would require either reproducible VGC absorption and GCV disposition or dosage adjustment based on therapeutic drug monitoring (TDM). We examined GCV population pharmacokinetics in solid organ transplant recipients receiving oral VGC, including the influence of clinical factors, the magnitude of variability, and its impact on efficacy and tolerability. Nonlinear mixed effect model (NONMEM) analysis was performed on plasma samples from 65 transplant recipients under VGC prophylaxis or treatment. A two-compartment model with first-order absorption appropriately described the data. Systemic clearance was markedly influenced by the glomerular filtration rate (GFR), patient gender, and graft type (clearance/GFR = 1.7 in kidney, 0.9 in heart, and 1.2 in lung and liver recipients) with interpatient and interoccasion variabilities of 26 and 12%, respectively. Body weight and sex influenced central volume of distribution (V_1 = 0.34 l/kg in males and 0.27 l/kg in females [20% interpatient variability]). No significant drug interaction was detected. The good prophylactic efficacy and tolerability of VGC precluded the demonstration of any relationship with GCV concentrations. In conclusion, this analysis highlights the importance of thorough adjustment of VGC dosage to renal function and body weight. Considering the good predictability and reproducibility of the GCV profile after treatment with oral VGC, routine TDM does not appear to be clinically indicated in solid-organ transplant recipients. However, GCV plasma measurement may still be helpful in specific clinical situations.

4.2. Introduction

Valganciclovir (VGC), a prodrug ester of ganciclovir (GCV) and L-valine, has been developed to overcome the poor oral bioavailability of GCV, which limits its exposure after oral administration. GCV administered as VGC is characterized by a 10-fold-higher oral bioavailability [6, 10, 19, 24], with a VGC dose of 900 mg once daily providing a systemic

exposure comparable to that of intravenous GCV at 5 mg/kg [6, 19, 24]. The efficacy of GCV delivered as VGC has been established for the prevention of cytomegalovirus (CMV) disease in kidney, heart, and kidney-pancreas recipients at high risk for developing it (i.e., CMV-seropositive donor/CMV-seronegative recipient [D+/R–]) [21] and recently for the treatment of CMV disease in organ transplant recipients [2].

After administration, VGC is absorbed by peptide transporters through the intestinal epithelium and hydrolyzed into GCV, which is only 1 to 2% bound to plasma protein [5] and extensively eliminated through the kidney by both glomerular filtration and tubular secretion. Thus, in renal insufficiency, the dosage of VGC has to be adjusted to the estimated glomerular filtration rate (GFR) [6]. GCV is secreted through the organic anion transporters (OAT) [14] and therefore at risk for drug interactions with transport inhibitors. Other factors could also influence VGC pharmacokinetics, including a patient's body weight (BW), gender, and comorbidities. Pharmacokinetic variability represents a potential nuisance for drug efficacy and safety, if it does not receive proper consideration on prescription. The potential burden of CMV infection plays a significant role after transplantation in terms of morbidity and mortality [8], and the incidence of infection is nonnegligible in CMV D+/R– patients in the absence of preventive treatment (45% according to Lowance et al. [18]). Thus, insufficient GCV exposure may lead to breakthrough viremia, especially in high-risk patients, or to the selection of resistant strains, as reported with oral GCV [16]. On the other hand, overexposure enhances the risk of dose-dependent hematologic toxicity [28]. The maintenance of circulating concentrations inside an effective and safe range is thus of therapeutic importance. This goal requires not only that dosages are adjusted to patient factors affecting VGC absorption and GCV disposition but also that VGC absorption and GCV disposition are sufficiently reproducible and predictable, knowing those factors. Otherwise, therapeutic drug monitoring (TDM) may represent a useful alternative to compensate for high interpatient variability [27].

The objectives of the present study were (i) to describe the population pharmacokinetic profile of GCV delivered as VGC and its variability in solid-organ transplant recipients receiving oral VGC for either oral VGC prophylaxis or treatment, (ii) to define clinical factors that could explain interpatient differences, and (iii) to explore the relation between GCV profile and efficacy and tolerability outcomes. Our findings thus help us to evaluate the usefulness of GCV TDM after VGC administration in solid-organ transplant patients.

4.3. Material and Methods

4.3.1. Patient population

This prospective observational study was conducted from November 2005 to January 2008 at the University Hospital of Lausanne (CHUV) and the University Hospital of Geneva (HUG) in Switzerland. Protocols were approved by local ethics committees. Adult solid-organ transplant patients at risk for CMV infection (donor or recipient CMV seropositive) receiving oral VGC prophylaxis, oral VGC treatment, or intravenous GCV treatment were enrolled consecutively after giving their written informed consent. Exclusion criteria were failure to provide informed consent or known intolerance to GCV or VGC. A 3-month course of VGC prophylaxis was administered from day 3 post-transplantation, except in lung transplant recipients that were donor seropositive and recipient seronegative who received VGC prophylaxis for 6 months. The VGC prophylactic dosage was 900 mg once daily in heart and lung recipients. Kidney transplant recipients, having on average a slight degree of renal impairment, received 450 mg once daily. Further dose adjustment to renal function was applied according to the manufacturer's recommendations. VGC therapeutic dosage for CMV disease [25] was 900 mg twice daily, adjusted to the renal function. Two patients had to receive intravenous GCV treatment. The dosage was of 5 mg/kg every 12 h, with further adjustment to renal function. GCV levels were measured monthly both at the trough point and 3 h after oral or intravenous administration during prophylaxis and at weekly intervals during treatment. During prophylaxis, the first sample was collected after at least 3 days of VGC administration, and the next one was given about 1 or 2 months later. Intensive pharmacokinetic data (rich data) obtained in two kidney recipients were also included in the analysis.

For each patient, the gender, height, age, graft type, CMV serostatus (both donor and recipient), and comorbidities were recorded. Samples were generally obtained under steady-state conditions (i.e., drug regimen unchanged for at least 3 days). However, when this condition was not reached, the detailed dosing schedule was recorded during the last 3 days. Actual dosing and sampling time information was carefully recorded on each sampling occasion, along with patient's BW, serum creatinine, concomitant medications, adverse events (nausea, diarrhea, skin toxicity, anemia, leucopenia, neutropenia, thrombocytopenia, and liver enzyme elevation). Adverse events were recorded as present or absent, including anemia (hemoglobin <10.4 g/l [male] or <9.9 g/l [female]), leucopenia (leukocytes <3.5 g/l), neutropenia (neutrophils <2.0 g/l), thrombopenia (platelets <140 g/l), and liver enzyme elevation (aspartate aminotransferase and alanine aminotransferase, >1.1 upper limit of normal range).

4.3.2. Analytical method

Blood samples (5.5 ml) were collected into Monovettes (Sarstedt, Nümbrecht, Germany), with K-EDTA as an anticoagulant. The samples were sent without delay to the laboratory, and plasma was isolated by centrifugation and stored at –20°C to ensure stability up to the time of analysis. Plasma GCV levels were determined by reversed-phase high-performance liquid chromatography coupled with spectrofluorimetric detection according to a validated method [22]. The calibration curve is linear between 0.1 and 10 mg/l. The mean absolute recovery of GCV was 100.3% ± 2.5%. The method is precise (with mean inter-day coefficients of variation [CVs] within 1.2% to 3.5%) and accurate (range of inter-day deviations, –0.4% to +1.4%).

4.3.3. Model-based pharmacokinetic analyses

The analysis was performed by using the NONMEM computer program written in FORTRAN 77 (version VI, with NM-TRAN version II) [3] running on a mainframe station (Sun Fire 3800 server with UltraSPARC III processors; Sun Santa Clara). It uses mixed (fixed and random) effects regression to estimate population means and variances of the pharmacokinetic parameters and to identify factors that influence them.

4.3.4. Structural model

The following stepwise procedure was used (see model selection below): first, one- and two-compartment models with first-order absorption from the gastrointestinal tract for oral VGC were compared based on the data from the two patients who underwent intensive kinetic investigation (rich data) and from the entire population (sparse data). The estimated parameters were the systemic clearance (CL), the intercompartmental clearance (Q), the central volume of distribution (V_1), the peripheral volume of distribution (V_2), and the absorption rate constant (k_a). Since GCV was administered intravenously only in two patients, the bioavailability (F) could not be estimated with sufficient accuracy and was fixed at 0.6 according to previous studies [6, 10, 19, 24]. Derived parameters were the absorption half-life [$t_{1/2a} = \ln(2)/k_a$] and the elimination half-life [$t_{1/2\beta} = \ln(2)/\lambda_\beta$, with λ_β derived form CL, Q, V_1, V_2].

4.3.5. Structural model

Exponential errors following a log-normal distribution were assumed for the description of interpatient variability of the pharmacokinetic parameters and were of the form , $\theta_j = \theta\, e^{\eta j}$ where θ_j is the individual pharmacokinetic parameter value in the jth individual, θ is the population parameter estimate and ηj is the random effect value, which is independently and normally distributed with a mean of 0 and variance ω. Considering potential modifications in patients' condition over time as a consequence of changes in pathophysiological processes (blood samples were drawn a few days to many months after transplantation), an inter-occasion variability [12] was assigned on clearance that accounted for three different occasions: up to month 1, up to month 2, and up to month 6 or more of the post-transplantation period. A specific model for time-varying creatinine clearance [30] was also tested. Proportional and combined proportional-and-additive error models were compared to describe intrapatient (residual) variability.

4.3.6. Covariate model

The covariate analysis was performed using a stepwise insertion/deletion approach. Visual inspection of the correlation between post hoc individual parameter estimates and the available covariates (demographic characteristics, comorbidities, and concomitant medications) was first conducted by graphical exploration. Potentially influential covariates were then incorporated sequentially into the pharmacokinetic model. The typical value of a given parameter θ (e.g., CL) was modeled to depend either linearly on the covariate *X* (general equation: $\theta = \theta_a * X$, where θ_a is the estimated coefficient) or as a power function for categorical covariates (general equation: $\theta = \theta_a * \theta_b{}^{X}$, where θ_a is the estimate of the basal value and θ_b is the contribution of the factor *X*). Covariates (*X*) evaluated for inclusion during the model building process were gender (sex), age, body weight (BW), height (HGT), GFR, comorbidities, and concomitant medications. Concomitant medications included the presence or absence of calcineurin inhibitors (ICAL) (tacrolimus or cyclosporine), mycophenolate (MMF), cotrimoxazole (COTM), and organic anion transporter inhibitors (OATI) [14]. Two different equations for the estimation of GFR were compared: (i) the traditional Cockroft-Gault equation, $GFR_{C\text{-}G}$ = [(150 – age) x BW]/Cr_s x 1.1 (if male) or x 0.9 (if female) [4], and (ii) the four-variable modification of diet in renal disease (MDRD) formula with individual body surface area, GFR_{MDRD} = 175 x $(Cr_s/88.4)^{-1.154}$ x $age^{-0.203}$ x 0.742 (if female) [15], where Cr_s is the standardized serum creatinine value expressed in µmol/l and

the age is given in years. This simplified four-variable MDRD formula was shown to accurately predict GFR in kidney transplant patients [7].
At the end of the analysis, all patient characteristics showing an influence on the parameters were again confirmed by comparing the full model (with all factors included) to models from which each of the factors was removed sequentially.

4.3.7. Model selection and parameter estimation

The models were fitted by use of the first-order conditional method (and 3 significant digits) with the subroutine ADVAN 4, TRANS 4. Goodness-of-fit statistics and graphical displays were used to compare models on each step of model building. The goodness-of-fit criterion was the change in the objective function (ΔOF) resulting from the addition of one covariate, which approximates a χ^2 distribution and can be regarded as statistically significant ($P < 0.05$) if it exceeds 3.8. A simulation based on the final pharmacokinetic estimates was performed with NONMEM using 1,000 individuals to calculate 95% prediction intervals of the concentrations versus time curve. Those individuals were taken as 70-kg male kidney transplant patients with GFR_{MDRD} of 50 ml/min/1.73m^2. The figures were generated with GraphPad Prism (version 4).

4.3.8. Concentration-effect analyses

Individual Bayesian estimates of the GCV trough concentration (C_{trough}) and the area under the curve (AUC) obtained through NONMEM were used to explore the relationship with prophylaxis outcomes (breakthrough viremia during prophylaxis and 3 months beyond) and tolerability (nausea, diarrhea, skin toxicity, anemia, leucopenia, neutropenia, thrombocytopenia, or liver enzyme elevation on sampling time). CMV viremia was detected by CMV DNA PCR [20] and recorded as either negative (limit of detection = 100 to 1,000 copies/ml depending on the cell count) or positive (limit of quantification = 1,000 copies/ml). Adverse events were recorded as present or absent based on the criteria presented above.
The relationship between GCV AUC and C_{trough} and those outcomes was assessed by using logistical regression analyses. A sample-level analysis (individual estimates) was complemented with a patient-level analysis (mean estimates) when significant, with a statistical significance level assigned at $P \leq 0.05$ for model improvement by the pharmacokinetic predictor (chi-square test, one-tailed distribution). Statistical analyses were performed using STATA software (version 8.2).

4.4. Results

4.4.1. Data

A total of 437 GCV plasma samples from 65 solid organ transplant patients were included in the population analysis (41 kidney, 10 heart, 12 lung, and 2 liver recipients). Blood samples were drawn from 55 patients receiving oral VGC prophylaxis (n = 330), from 5 patients receiving oral VGC treatment for CMV infection or disease (n = 52), from 3 patients receiving both successive regimens (n = 23 and 26, respectively), and from 2 patients receiving intravenous GCV treatment for CMV disease (n = 6). Eight patients were not enrolled due to transfer to another hospital or refusal. A median (range) of 6 (1 to 22) samples per subject were available. In addition to this sparse sampling data set, four full concentration-time profiles at steady state were available from two patients under VGC 450 mg once daily (n = 26, six to seven time points per profile before the dose and from 2 to 24 h after drug intake: rich data set). Among the 437 GCV samples used for model building, 197 (45%) were collected up to 6 h after dosing, 46 (11%) were obtained between 6 and 14 h, 168 (38%) were obtained between 14 and 26 h after dosing, and the remaining 26 (6%) were collected later than 26 h after drug intake (with a maximum of 75 h). Table 1 lists the patients' characteristics.

4.4.2. Population pharmacokinetic analyses

The model-building process (structure, variability) is shown in table 2. A two-compartment model with first-order absorption from the gastrointestinal tract appropriately described the data (both rich and sparse) (ΔOF = –37.5). Since GCV is known to be almost exclusively eliminated by the kidney, $GFR_{C\text{-}G}$ was introduced as a covariate on CL at an early step, improving significantly the description of the data (ΔOF = –200.9). An interpatient variability was best assigned to both CL and V_1. The use of a proportional plus additive error model for the residual intrapatient variability was the most satisfactory at this early step.

The pharmacokinetic estimates and the variability (CV%) of the population model, with only $GFR_{C\text{-}G}$ as covariate on systemic clearance, were as follows: CL = 1.35 * GFR l/h (26%), Q = 3.1 l/h, V_1 = 28 l (28%), V_2 = 19.5 l, k_a = 0.65 h^{-1} and F_{fixed}= 0.6.

Table 1: Characteristics of patients evaluated in the population pharmacokinetic analysis of valganciclovir

Patients (n=65)	**Number**	**% of total or (range)**
Baseline characteristics		
Sex (men / women) (no.)	45/20	69/31
Median age (years)	55	(18 – 70)
Median body weight (kg)	72	(46 – 115)
Median height (cm)	172	(147 – 192)
Median creatinine (µmol/l)	108	(29 – 691)
Graft type (no. of patients)		
Kidney	41	63
Heart	10	15
Lung	12	18
Liver	2	3
CMV serostatus (no. of patients)		
D+/R-	22	34
D+/R+	28	43
D-/R+	15	23
Comorbidity (no. of patients)		
Cardiopathy	19	29
Overweight	9	14
Cystic fibrosis	1	2
Samples (n=437)		
Drug (no. of samples)		
VGC: - CMV prophylaxis	353	81
- CMV therapy	78	18
GCV: - CMV therapy	6	1
Concomitant medications		
Tacrolimus/Cyclosporine (ICAL)	347/90	79/21
Mycophenolate (MMF)	374	86
Cotrimoxazole (COTM)	321	74
OAT inhibitors (OATI)	354	73

The model-building steps for the covariate analysis are detailed in table 2. A time-varying creatinine clearance [30] did not improve the model. Inclusion of the covariates sex (ΔOF = –10.5) and graft type (ΔOF = –34.1) as modifiers of CL in addition to $GFR_{C\text{-}G}$ significantly improved the model. CL differed between female and male patients by 23%, which fairly corresponds to the correction factor for sex included in the Cockroft-Gault formula. The assignment of GFR_{MDRD} rather than $GFR_{C\text{-}G}$ with the addition of the covariate sex on CL reduced the objective function (ΔOF = –16.4), with a difference of 24% remaining between females and males. The type of graft had a significant influence on CL, showing a 40 and 13% lower elimination of GCV in heart and lung/liver transplant patients, respectively, compared to kidney transplant recipients. Among the concomitant medications potentially influencing CL, only ICAL (ΔOF = –16.6) significantly improved the fit, showing a reduction in CL of 20% in patients receiving cyclosporine versus those under tacrolimus treatment.

Inclusion of the demographic covariates (body weight, sex, height) on V_1 significantly improved the pharmacokinetic model with a predominant effect from weight (ΔOF= -30.4). Multivariate confirmation of the significant covariates showed that only GFR, sex and graft type remained statistically significant regarding CL (ΔOF= -46.7) and only body weight and sex remained statistically significant regarding V_1 (ΔOF= -35.6).

The introduction of a supplemental term for interoccasion variability, accounting for changing clearance over time from graft, improved significantly the fit (ΔOF= -62.1). The proportional interpatient and interoccasion variability terms were 23 and 14%, respectively. At this last step, only the proportional error remained significant, while the additive component vanished out.

The parameter estimates for the final model are given in table 3. Derived parameters were an absorption half-life ($t_{1/2a}$) of 1.2 h and a median elimination half-life ($t_{1/2\beta}$) of 8 h (range, 5 to 68 h). Figure 1 shows the overall goodness-of-fits plots and the concentration-time plot of the 36 kidney transplant recipients under VGC at 450 mg once daily, along with the average population prediction and 95% prediction interval for male kidney transplant patients receiving VGC at 450 mg once daily (GFR_{MDRD} of 50 ml/min, body surface of 1.73 m^2, and BW of 70 kg).

Table 2: Summary of the model-building steps of the population pharmacokinetic analysis of valganciclovir[a]

Hypothesis[a]	Model	θ_a	θ_b	θ_c	θ_d	θ_e	θ_f	ΔOF[b]	OF[c]
Model structure									
1-compartment, 1st order	ω(CL)								-4349.5
2-compartment, 1st order	ω (CL)							-37.5	-4386.9
Model variability (ω)									
GFR_{C-G} on CL, variability on CL	$CL = \theta_a * GFR_{C-G}$ ω(CL)	1.41						-200.9	-4587.8
and variability on V_1	**$CL = \theta_a * GFR_{C-G}$ ω(CL, V_1)**	1.41			21.6			-20.3	-4608.2
and variability on V_1 & Q	$CL = \theta_a * GFR_{C-G}$ ω(CL, V_1, Q)	0.83			8.6			69.3	-4538.8
and variability on V_1 & V_2	$CL = \theta_a * GFR_{C-G}$ ω(CL, V_1, V_2)	1.32			14.5			-3.3	-4611.5
Covariate analysis									
Four-variable MDRD estimated GFR	$CL = \theta_a * GFR_{MDRD}$	1.52						-2.9	-4899.7
Extra-renal clearance?	$CL = \theta_a * GFR_{C-G} + \theta_b$	1.35	0.015					+0.8	-4896.0
Does sex influence CL (GFR_{C-G})?	$CL = \theta_a * GFR_{C-G} * \theta_b^{sex}$ (sex=0 if male, =1 if female)	1.27	1.23					-10.5	-4907.3
Does sex influence CL (GFR_{MDRD})?	$CL = \theta_a * GFR_{MDRD} * \theta_b^{sex}$ (sex=0 if male, =1 if female)	1.43	1.24					-16.4	-4913.2
Does ICAL influence CL?	$CL = \theta_a * GFR_{C-G} * \theta_b^{ICAL}$	1.40	0.79					-16.6	-4913.4
Does MMF influence CL?	$CL = \theta_a * GFR_{C-G} * \theta_b^{MMF}$	1.36	0.99					0.0	-4896.8
Does OATI influence CL?	$CL = \theta_a * GFR_{C-G} * \theta_b^{IOAT}$	1.37	0.98					-0.3	-4897.1
Does COTM influence CL?	$CL = \theta_a * GFR_{C-G} * \theta_b^{COTM}$	1.30	1.06					-3.4	-4900.2
Does cardiopathy influence CL?	$CL = \theta_a * GFR_{C-G} * \theta_b^{Card}$	1.33	1.05					-0.5	-4897.3
Does graft type influence CL?	$CL = \theta_{graft} * GFR_{C-G}$ (K: θ_a, H: θ_b, Lu/Li: θ_c)	1.50	0.90	1.31				-34.1	-4930.9
Does BW influence V_1?	$V_1 = \theta_d * (BW/70)$				30.0			-30.4	-4927.2
Does sex influence V_1?	$V_1 = \theta_d * \theta_e^{Sex}$ (sex=0 if male, =1 if female)				28.7	0.64		-16.0	-4912.8
Does BW & sex influence V_1?	$V_1 = \theta_d * (BW/70) * \theta_e^{Sex}$ (sex=0 if male, =1 if female)				27.7	0.79		-5.3	-4932.4
Does HGT influence V_1?	$V_1 = \theta_d * (HGT/170)$				29.2			-6.7	-4903.5
Does BW & HGT influence V_1?	$V_1 = \theta_d * (BW/70) * (HGT/170)$				26.4			-1.5	-4928.6
Does graft type influence V_1?	$V_1 = \theta_{graft} * (BW/70)$ (K: θ_d, H: θ_e, Lu/Li: θ_f)				30.1	29.6	30.2	-30.4	-4927.2
Simple Model	**$CL = \theta_a * GFR_{C-G}$**	1.35							-4927.2
	$V_1 = \theta_d * (BW/70)$				30.0				
Inter-occasion variability (IOV) on CL	$CL = \theta_a * GFR_{C-G}$	1.39						-62.1	-4989.3
	$V_1 = \theta_d * (BW/70)$				23.2				
Final Model with IOV	**$CL = \theta_{graft} * GFR_{MDRD}$ (K: θ_a, H: θ_b, Lu/Li: θ_c) * θ_d^{sex}**	1.68	0.86	1.17	1.21			-66.4	-5055.7
	$V_1 = \theta_e * (BW/70) * \theta_f^{Sex}$					24.0	0.72		

[a] GFR_{C-G}, GFR estimated with Cockroft-Gault formula (l/h); ICAL (tacrolimus = 0, cyclosporine = 1); OATI, OAT inhibitors; Card, cardiopathy; K, kidney recipients; H, heart recipients; IOV, interoccasion variability; Lu/Li, lung and liver recipients; GFR_{MDRD}, GFR estimated with four-variable MDRD formula (l/h); BW, body weight (in kg); HGT, height (in cm).
[b] △OF, difference in the NONMEM objective function compared to the best previous model.
[c] OF, NONMEM objective function.

Table 3: Population pharmacokinetic parameter estimates of valganciclovir

Parameter[a]	Mean estimate (SE)	
	Population (%)[b]	% Variability[c]
F	0.6 (fixed)	
k_a (h^{-1})	0.56 (19)	
CL (l/h) = $\theta_{GraftType} \cdot GFR_{MDRD}$ (l/h)$\cdot \theta_{female}^{sex}$		26 (54)*
θ_{kidney}	1.68 (5.5)	
θ_{heart}	0.86 (14)	
$\theta_{lung/liver}$	1.17 (9.0)	
θ_{female} (male: sex = 0, female: sex = 1)	1.21 (8.3)	
V_1 (l) = $\theta_{BW} \cdot$[BW (kg)/70 kg]$\cdot \theta_{female}^{sex}$		20 (75)*
θ_{BW}	24 (12)	
θ_{female} (male: sex = 0, female: sex = 1)	0.78 (9.7)	
Q (l/h)	4.1 (19)	
V_2 (l)	22 (7.4)	
IOV (CV %)		12 (54)[†]
σ_{prop} (CV %)		21 (41)[‡]

[a] F, bioavailability; GFR_{MDRD} four-variable MDRD estimated GFR; BW, body weight. The other abbrviations are as defined in the text. The IOV on CL is expressed as the CV (%). The residual variability (σ_{prop}) in the GCV plasma concentrations was associated with the proportional error term and is expressed as CV (%).
[b] The standard errors (SE) of the estimates, calculated as $SE_{estimate}$/estimate, expressed as a percentage, are given in parentheses.
[c] Estimates of variability are expressed as the CV (%). *, Interpatient;[†], interoccasion;[‡], residual. Standard errors (SE) of the variance components, calculated as $\sqrt{SE_{estimate}}$/estimate, are expressed as a percentage.

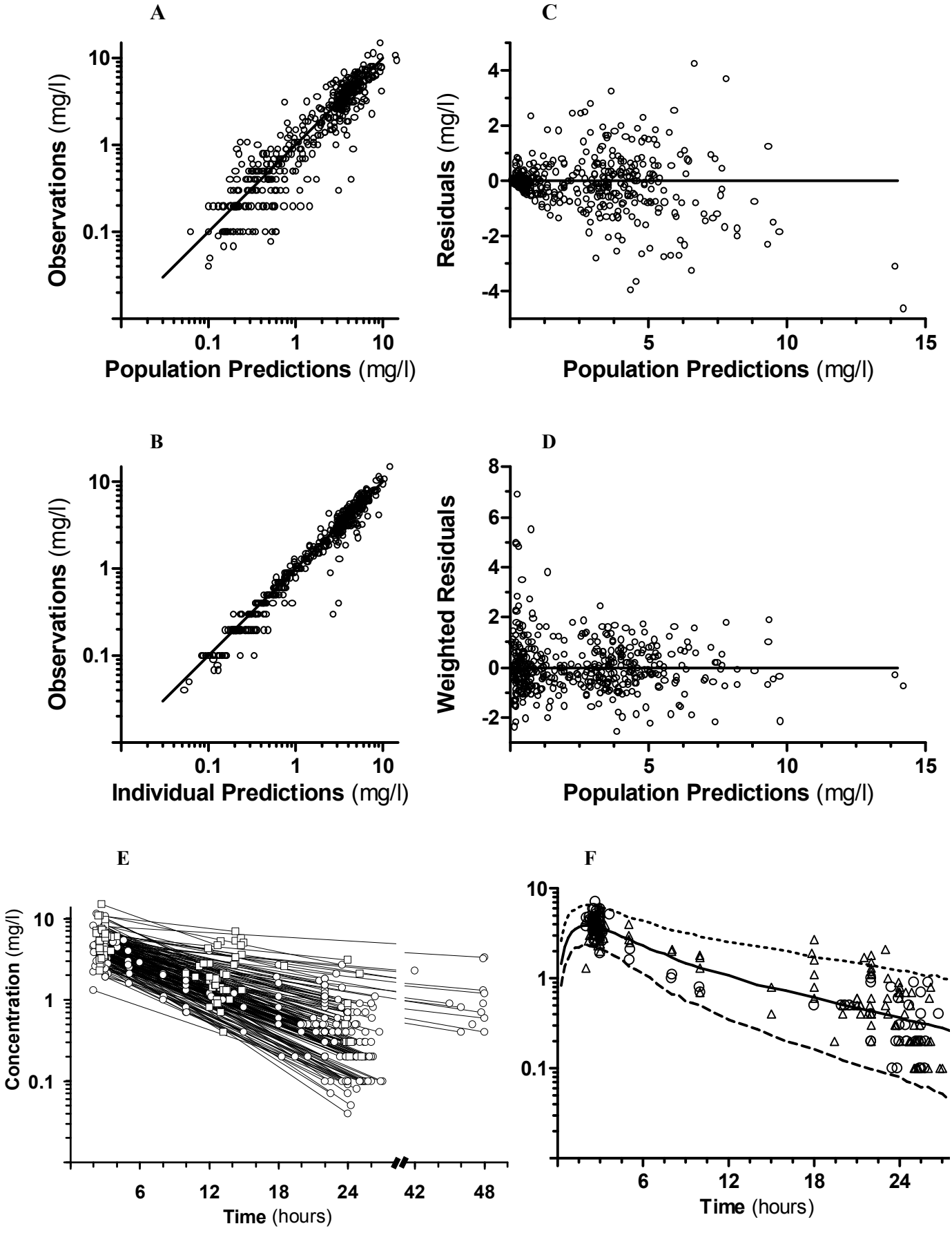
A
Observations (mg/l)
Population Predictions (mg/l)
C
Residuals (mg/l)
Population Predictions (mg/l)
B
Observations (mg/l)
Individual Predictions (mg/l)
D
Weighted Residuals
Population Predictions (mg/l)
E
Concentration (mg/l)
Time (hours)
F
Time (hours)

Figure 1: Goodness-of-fit plots of the final model for valganciclovir. (A) Log-log plot of observed concentrations versus population predictions. The line indicates the line of identity. (B) Log-log plot of observations versus individual predictions. The line indicates the line of identity. (C) Population residuals versus population predictions. The line is at ordinate value zero. (D) Population weighted residuals versus population predictions. The dotted line is at ordinate value zero. (E) Plasma concentrations in 65 solid-organ transplant recipients receiving valganciclovir prophylaxis (open circles) or treatment (open squares) of CMV infection/disease. (F) Pharmacokinetic profile of ganciclovir in a selection of 36 kidney transplant recipients under valganciclovir at 450 mg once daily (men: open triangles, women: open circles) with average population prediction (solid line) and a 90% prediction interval (dashed lines) for male kidney transplant patients (GFR of 50 ml/min, body surface area of 1.73 m^2, body weight of 70 kg).

4.4.3. Prophylactic efficacy and tolerability

Viremia was monitored during the 3-month prophylaxis and a further 3-month follow-up in 49 and 41 patients, respectively. During prophylaxis, CMV viremia was detected in three (6%) patients (one lung [D+/R–] and two heart [D+/R– and D+/R+] recipients); however, this did not exceed a low level (<100 copies/ml). No association between estimates of mean GCV exposure (AUC) or trough concentration and breakthrough viremia was noticed (P = 0.4 and = 0.2, respectively [chi-square test]). During the 3 months after prophylaxis cessation, CMV viremia was detected in 13 of 41 (32%) transplant recipients, including 2 patients (D+/R–) with CMV disease who were treated with oral VGC. The remaining 11 patients had low-grade viremia and were not treated with VGC. No association was observed either between estimates of GCV AUC or C_{trough} and after prophylaxis viremia (P = 0.6 and = 0.7, respectively).

Regarding VGC prophylaxis and tolerability, nausea/vomiting was reported in 7% of patients, diarrhea was reported in 18% of patients, skin toxicity (nonserious) was reported in 9% of patients, anemia in 67%, leucopenia was reported in 14% of patients (neutropenia in 9% of patients), thrombocytopenia was reported in 16% of patients, and liver enzyme elevation was reported in 25% of patients. Per-sample analyses indicated a significant association between estimates of GCV AUC and the occurrence of anemia, neutropenia, and leucopenia and between C_{trough} and diarrhea (P = 0.004 [chi-square test]). However, these associations did not retain a statistically significant level in per-patient analyses, except C_{trough} and diarrhea (P = 0.009). Considering only patients under VGC prophylaxis, no significant association remained between C_{trough} and the occurrence of diarrhea.

4.5. Discussion and conclusion

Oral VGC is currently supplanting GCV in most indications, including the prophylaxis of CMV infection in solid-organ transplant patients and the treatment of overt CMV infection. According to the manufacturer, VGC (similar to GCV) is to be adjusted to renal function.

However, graft recipients most often receive the standard dosage regimen without regard to the type of organ transplant, BW, sex, associated comorbidities, and medications. Notably, a normal renal function will never be fully restored in most kidney transplant recipients. The characterization of VGC pharmacokinetic profile, the identification of influential covariates beyond GFR and the quantification of interpatient and intrapatient variability are important elements for evaluating the potential usefulness of more elaborated dosage adjustment recommendations, including a TDM strategy.

The pharmacokinetic results of our population analysis are in agreement with other recently reported estimates [1, 31]. Derived parameters such as absorption and elimination half-life are also congruent with previous observations [1, 6, 31]. Our population of patients is characterized by a wide range of renal function (GFR_{C-G} range, 10 to 170 ml/min), leading to a comparable range of elimination half-life values.

The dominant influence of renal function on GCV clearance has been reported in previous population analyses [1, 6, 31]. GFR can be estimated with the traditional Cockroft-Gault formula and by the MDRD formula, which has shown some advantages in renal transplant patients [26]. A difference in clearance between male and female patients was observed beyond the factor of correction for sex included in both formulas and the calculation for individual body surface area. The higher clearance observed in females could be due to sex-related differences in OAT expression, as reported in the rat and mouse [9, 17]. Our model includes a small but significant difference in the correlation between GFR and GCV clearance according to graft type. This effect could be explained by the difference in the patients' drug regimens between types of transplantation. For example, the administration of an ICAL affected clearance significantly in the univariate analysis, but no more than when it was combined with graft type; noticeably, cyclosporine was almost exclusively received by heart transplant recipients, this drug being known to decrease effective renal plasma flow to a slightly larger extent than tacrolimus, for the same degree of immunosuppressive effect [13]. Regarding concomitant medication, both MMF and trimethoprim have been shown to reduce GCV renal clearance [11, 33]. Although both drugs were administered to a large proportion of our population of patients (in 86 and 73% of samples, respectively), no effect of MMF on the GCV pharmacokinetic profile could be detected. COTM, which was specifically investigated in two kidney transplant recipients, also did not show such an effect (data not shown). A large proportion of our patients received various agents reported as OAT inhibitors (such as omeprazole, acetylsalicylic acid, etc. [14]) that did not affect GCV CL in our analysis, probably because several redundant anion transporter systems exist in the kidney [29].

The interpatient variability in GCV clearance and the volume of distribution estimated in the present study are in accordance with recently reported data [1, 31], despite the inclusion of an interoccasion variability term in our model. An interoccasion variability was justified considering the sampling schedule for patients under VGC prophylaxis, with a first blood sample collected during the first week post-transplant and subsequent samples collected after 1 and 2 months.

Interpatient differences in oral absorption were not specifically identified during the NONMEM analysis and were therefore combined with interpatient variability in clearance and volume (since bioavailability was fixed in our model). The variability in both of those parameters remained limited, however (26 and 20%, respectively), meaning that the absorption and disposition profile of VCG was fairly reproducible and predictable in our population of transplant recipients.

Our pharmacodynamic analysis did not reveal any significant association between GCV exposure (AUC) or trough concentration and the occurrence of breakthrough viremia during prophylaxis or during the following 3 months after prophylaxis discontinuation. In a previous population pharmacokinetic-pharmacodynamic study including 240 D+/R– solid-organ transplant recipients, GCV systemic exposure appeared to correlate with antiviral activity in terms of the incidence of developing CMV viremia during prophylaxis (3 months post-transplantation) and for the following month [32]. Among our population of D+/R– patients, 14% developed detectable low-grade CMV viremia during prophylaxis (compared to 12% reported by Wiltshire et al. [32]). Despite this comparable incidence, our analysis was certainly limited by the small number of patients. GCV needs to be bioactivated in cells into GCV triphosphate to inhibit virus replication. The GCV plasma concentration is thus only a surrogate of the actual active-form concentration, explaining the loose concentration-effect relation.

Our analysis did not detect significant relationships between GCV exposure or trough concentration and the occurrence of adverse events. Wiltshire et al. [32] reported a weak tendency toward increased neutropenia and leucopenia with high GCV exposure, but no association with anemia. Here again, our study population was probably inadequate to assess concentration-toxicity relationships. The identification of diarrhea as a concentration-related side effect was probably associated with patients treated for CMV disease (including some with CMV colitis) with a high dose of VGC. The occurrence of diarrhea may be related to a high dose of VGC and/or CMV colitis, a confounding factor that could not be circumvented. Taking into account all these elements, a routine clinical pharmacokinetic monitoring of VGC in solid organ transplant recipients cannot be expected to be of much benefit. Nevertheless, in our experience, selective TDM of VGC appeared to be useful in

specific clinical cases (e.g., unstable or not assessable renal function or continuous renal replacement therapy [23], iterative hemodialysis, and an unexplained absence of treatment response).

In conclusion, the pharmacokinetic parameters of VGC in a population of solid-organ transplant patients were adequately described by our population model, confirming a predominant role for renal function in clearance and for BW in the central volume of distribution. The type of transplant and gender also influenced clearance and the central volume of distribution, respectively. No drug interactions were found to impact VGC disposition. Only a limited degree of interpatient variability remained unexplained, which suggests a minor effect of additional unidentified covariates (e.g., genetic influences and absorption issues). Residual variability was moderate as well. Efficacy outcomes, as well as the occurrence of adverse events, did not correlate with drug exposure. This analysis highlights the importance of thorough adjustment of VGC dosage to renal function and BW. Considering the good predictability and reproducibility of the GCV profile after the administration of oral VGC in solid organ transplant recipients, routine TDM does not appear to be clinically indicated. However, GCV plasma measurement may still be helpful in specific clinical situations.

4.6. References

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Chapter 5 : Clinical applications

Presentation of chapter 5

Which was the ganciclovir exposure associated with effective treatment of CMV disease? Did some patients fail to respond due to low ganciclovir levels?

Which recommendations are to be given regarding patients under valganciclovir prophylaxis while receiving continuous renal replacement therapy?

The following chapter is based on two articles reporting clinical applications of ganciclovir concentration measurement.

First, ganciclovir levels measured in patients with various responses to CMV disease treatment are presented. This limited set of observations is issued from our patients population (chapter 4). The pharmacokinetic analysis was used to compare ganciclovir exposure among those patients, with respect to their clinical response.

In the second part, the poorly covered question of drug adjustment in continuous renal replacement therapy is addressed for valganciclovir. Although some authors had previously described ganciclovir disposition in such conditions, none reported valganciclovir disposition.

Variable viral clearance despite adequate ganciclovir plasma levels during valganciclovir treatment for cytomegalovirus disease in D+/R- transplant recipients

N. Perrottet[1], M. Oriol[2], F. Lamoth[2], J.P. Venetz[2], R. Shali[3], L.A. Decosterd[1], T. Buclin[1], M. Pascual[2], P. Meylan[3]

[1] Division of Clinical Pharmacology and Toxicology, University Hospital (CHUV), Lausanne, Switzerland

[2] Organ Transplantation Centre, University Hospital (CHUV), Lausanne, Switzerland

[3] Microbiology Institute, University Hospital (CHUV), Lausanne, Switzerland

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Related appendices: 5.1 and 5.2

5.1. Variable viral clearance despite adequate ganciclovir plasma levels during valganciclovir treatment for cytomegalovirus disease in D+/R- transplant recipients

5.1.1. Abstract

Background: Valganciclovir, the oral prodrug of ganciclovir, has been demonstrated equivalent to iv ganciclovir for CMV disease treatment in solid organ transplant recipients. Variability in ganciclovir exposure achieved with valganciclovir could be implicated as a contributing factor for explaining variations in the therapeutic response. This prospective observational study aimed to correlate clinical and cytomegalovirus (CMV) viral load response (DNAemia) with ganciclovir plasma concentrations in patients treated with valganciclovir for CMV infection/disease.

Methods: Seven CMV D+/R- transplant recipients (4 kidney, 2 liver and 1 heart) were treated with valganciclovir (initial dose was 900-1800 mg/day for 3-6.5 weeks, followed by 450-900 mg/day for 2-9 weeks). DNAemia was monitored by real time quantitative PCR and ganciclovir plasma concentration was measured at trough (C_{trough}) and 3h after drug administration (C_{3h}) by HPLC.

Results: Four patients presented with CMV syndrome, two had CMV tissue-invasive disease after prophylaxis discontinuation, and one liver recipient was treated pre-emptively for asymptomatic rising CMV viral load 5 weeks post-transplantation in the absence of prophylaxis. CMV DNAemia decreased during the first week of treatment in all recipients except in one patient (median decrease: -1.2 log copies/ml, range: -1.8 to 0) despite satisfactory ganciclovir exposure (AUC_{0-12} = 48 mg·h/l, range for the 7 patients: 40-118 mg·h/l). Viral clearance was obtained in five patients after a median of time of 34 days (range: 28-82 days). Two patients had recurrent CMV disease despite adequate ganciclovir exposure (65 mg·h/l, range: 44-118 mg·h/l).

Conclusions: Valganciclovir treatment for CMV infection/disease in D+/R- transplant recipients can thus result in variable viral clearance despite adequate ganciclovir plasma concentrations, probably correlating inversely with anti-CMV immune responses after primary infection.

5.1.2. Introduction

Cytomegalovirus (CMV) used to rank first as a cause for morbidity and mortality among solid organ transplants (SOT) recipients [1]. CMV disease can be prevented either by CMV prophylaxis or pre-emptive treatment guided by CMV viral load monitoring [1]. CMV–seronegative recipients who receive a transplant from a CMV-positive donor (D+/R-) are at highest risk of developing late CMV disease despite prophylaxis [2] with an incidence up to 43% [3]. Valganciclovir, the ester prodrug of ganciclovir, is currently used for CMV prophylaxis [4] and has been demonstrated equivalent to iv ganciclovir for CMV disease treatment in SOT recipients [5]. As ganciclovir plasma levels were not reported in most treatment studies using valganciclovir, variability in ganciclovir exposure achieved with this oral prodrug (e.g. due to malabsorption) could be imagined as a contributing factor partly explaining variations in the therapeutic response. The present prospective study aimed at describing the clinical and virological outcome (CMV viral load response) along with ganciclovir plasma concentration exposure in SOT patients receiving valganciclovir treatment for CMV infection/disease.

5.1.3. Patients and methods

Patients

The present consecutive series of patients presenting CMV disease and treated with valganciclovir was observed during a population pharmacokinetic study of valganciclovir conducted at the University Hospital, Lausanne, Switzerland, with the approval of the local ethics committee [6]. Adult SOT recipients with either CMV asymptomatic infection treated pre-emptively or with CMV disease [7] receiving oral valganciclovir treatment were enrolled after giving a written informed consent. Valganciclovir therapeutic dosage for CMV infection was 900 mg twice daily (adjusted to renal function according to the manufacturer recommendations and subsequently to ganciclovir blood levels) followed by a maintenance therapy (900 mg once daily with similar adjustment). Ganciclovir levels were measured weekly at trough (C_{trough}) and 3 hours after oral administration (C_{3h}) during treatment along with CMV viral load. The duration of the therapy was left to the decision of the physician in charge of the patient. In case of recurrence of CMV disease (defined as second episode after first CMV disease symptoms resolution), valganciclovir was reintroduced at therapeutic dosage. Kidney and heart transplant recipients received induction therapy with basiliximab (n=3) or thymoglobuline (n=2). The maintenance immunosuppressive regimen included prednisone in all patients associated with either tacrolimus (in 5 patients) or cyclosporine (in

2 patients), and mycophenolate mofetil (in 4 patients), mycophenolate sodium (in 1 patient) or azathioprine (in 1 patient).

Ganciclovir plasma level and pharmacokinetic profile

Plasma ganciclovir concentrations were determined by reverse-phase HPLC coupled with spectrofluorimetric detection according to a validated method [8]. The calibration curve was linear between 0.1 and 10 mg/l, the inter-day coefficient of variation was lower than 3.5% and the range of inter-day deviations comprised within –0.4 to +1.4%.

Ganciclovir plasma concentration results were analysed in a population pharmacokinetic study in the whole population of 65 transplant patients including this subgroup along with a majority of patients receiving valganciclovir for CMV prophylaxis. The analysis was performed by non-linear mixed effect modelling using the NONMEM® computer program. The structural model was two-compartment with first-order absorption. Systemic clearance was markedly influenced by GFR, sex and graft types. Body weight and sex influenced central volume of distribution. There was no difference in drug disposition between patients receiving valganciclovir for prophylaxis versus therapy. Ganciclovir exposure was evaluated by calculating the area under the curve (AUC) for each individual and sampling occasion, based on the subject-specific clearance value estimated at the end of the population analysis (maximum likelihood a posteriori Bayesian estimation) [6].

Virological monitoring

CMV viremia

CMV viremia was measured in whole blood using a CMV DNA real time quantitative PCR [9] with results expressed in number of copies/ml (limit of quantification: 1000 copies/ml, threshold of detection close to 100 copies/ml). DNAemia clearance was defined after one negative PCR. Recurrence of CMV disease was defined as reappearance in the blood of CMV DNA accompanied by symptoms.

CMV antibody

CMV antibody status of donor and recipient was determined for anti-CMV IgG by enzyme-linked fluorescent assay (ELFA, reference value: 4-6 EU/ml) (Vidas, BioMérieux, Marcy l'Etoile, France) and for EBV IgG by Viral Capsid Antigen immunofluorescence (VCA IF, reference value: titre 20) (Merifluor® EBV IgG IFA-IFT, Meridian Bioscience, Ohio, USA). Recipient CMV antibody were assessed for some patients during and after the treatment for IgM by enzyme immunoassay (EIA, reference value: index 0.9-1.1) (CMV-IgM-EIA test,

Medac, Hambourg, Germany) and IgG by ELFA. EBV antibody were measured in one EBV D+/R- patient for IgM by VCA IF (reference value: titre 10) or by bead array immunoassay (VCA BAIA, reference value: 100-120 UA/ml) (AtheNA Multi-Lyte, Zeus Scientific, NJ, USA) and for IgG by VCA IF or by VCA BAIA (reference value: 100-120 UA/ml).

CMV and EBV T-cell response

CMV specific and EBV specific T-cell response were assessed only in a single patient using interferon-gamma (IFN-γ) enzyme linked immunospot (ELISPOT, limit of detection: 55 SFU/mio cells) (Becton and Dickinson company, NJ, USA).

Genotypic sensitivity testing

Mutations in the CMV UL97 kinase gene were looked for in one patient. DNA was purified with the MagNApure LC instrument according to the manufacturer (Roche, magNApure DNA kit I) from either 200 µl whole blood or from an infected fibroblast cell culture inoculated with a gastric biopsy. Part of the UL97 region covering most of the known mutations associated with resistance to ganciclovir (codons 437-609) [10,11] was amplified by PCR using CMV_UL97M_F (TGCACGTTGGCCGACGCTAT: position 1308-1327 within the UL97 open reading frame) and CMV_UL97M_R (GCCGCCAGAATGAGCAGACA position 1837-1818 on the complementary strand of the UL97 open reading frame). PCR was done with 200 nmol/l each primer and 5 µl DNA in a 50 µl reaction containing 1.25 units Amplitaq Gold, 1.5 mmol/l MgCl2 in 1 x PCR buffer II (Applied Biosystems) supplemented with 4% Dimethyl sulfoxyde (Sigma), with the following cycling profile: 95°C for 9 min. followed by 40 cycles (95°/30" 58°/1'30" 72°/2') and a final extension step of 5' at 72°C.

Amplified DNA was analysed on a 2% agarose gel in the presence of ethidium bromide and the 530 base pairs amplicon purified with a PCR purification kit according to the manufacturer (Qiagen). DNA was then sequenced with the Big Dye terminator (v.1.1) chemistry according to the manufacturer (Applied Biosystems) using either PCR primer and subjected to capillary electrophoresis in an ABI3130XL instrument. Sequences were assembled from both strands and compared to the Genbank non redundant database using BLAST (Basic Local Alignment Search Tool, http://www.ncbi.nlm.nih.gov/blast/Blast.cgi).

5.1.4. Results

Seven CMV D+/R- patients (4 kidney, 2 liver and 1 heart recipients) were treated with valganciclovir: 6 for late-onset CMV disease (CMV syndrome n=4 and CMV tissue-invasive disease n=2) and one pre-emptively for CMV asymptomatic infection (n=1) (table 1). Treatment started between 1.4 and 21.5 months after discontinuation of valganciclovir prophylaxis in 6 patients and 5 weeks after transplantation in the liver recipient who was

treated pre-emptively. Initial valganciclovir dose was 900-1800 mg/day adjusted to calculated creatinine clearance for a median of 38 days (range: 20-63 days), followed by maintenance therapy (450-900 mg/day for a median of 27 days (range: 0-62 days). During valganciclovir treatment, maintenance immunosuppressive regimen consisted in prednisone 5-15 mg per day (depending on the time post-transplantation), in tacrolimus (mean plasma trough levels between 6-10 µg/l) in 5 patients and cyclosporine (mean plasma trough levels between 170-250 µg/l) in 2 patients. Doses of mycophenolate mofetil were reduced in 4 patients (initially 1-2 grams per day to 0.5-1 gram per day when valganciclovir was initiated and further stopped in 2 patients), as well as mycophenolate sodium in 1 patient (360 mg per day then stopped) and azathioprine in 1 patient (150 mg per day progressively reduced over 2 months then stopped). Figures 1 and 2 show valganciclovir dosage, ganciclovir plasma levels and CMV viral load over time for each patient.

Table 1: Patients characteristics, timing and type of CMV infection/disease and of recurrence

#	Age	Sex	Graft type *date*	Diagnostic	CMV Prophylaxis	Interval[a] months	CMV disease	Log CMV DNA change /1st week	Clearance of CMV viremia (Time to clearance)	Recurrence of CMV disease *(time from end of therapy)*	Immunosuppressive maintenance regimen[b]
1	64	M	Liver *19.02.2005*	Cirrhosis Child C Alpha-antitrypsin deficiency	VGC[c] 450 mg bid 6 months	8.9	Colitis (definite)	-1.2	Yes *(34 days)*	No	tacrolimus (10 µmol/l)[d] prednisone (7.5 qd) MMF[e] (stop for 1.5 months)
2	53	F	Cardiac *26.05.2005*	Idiopathic dilated cardiomyopathy	VGC 450 mg bid 3 months	6.2	Colitis (definite)	-1.3	No	No	cyclosporine (200 µmol/l)[d] prednisone (10 mg qd) MMF[e] (1000 mg bid)
3	46	M	Kidney *11.10.2004*	Drug toxicity: cisplatin, ifosfamide, contrast products, non steroidal analgesics	VGC 450 mg qd 3 months	24.5	Syndrome (probable)	-1.8	Yes *(29 days)*	No	tacrolimus (7 µmol/l)[d] prednisone (5 mg qd) MPS[f] (180 mg bid)
4	62	M	Kidney *24.03.2006*	Hepatorenal polykystosis	VGC 450 mg qd 3 months	9.4	Syndrome (probable)	-1.2	No	Syndrome (probable) *(21 days)* Colitis (definite) *(45 days)*	tacrolimus (8 µmol/l)[d] prednisone (5 mg qd) MMF[e] (500 mg bid)
5	49	M	Liver *03.07.2007*	Cirrhosis Child A Hepatocellular carcinoma (HCV)	None	1.4	Asymptomatic infection	-0.8	Yes *(28 days)*	No	cyclosporine (240 µmol/l)[d] prednisone (15 mg qd) AZA[g] (150 mg qd)
6	64	M	Kidney *13.12.2006*	Hepatorenal polykystosis	VGC 450 mg qd 3 months	9.7	Syndrome [h] (probable)	-0.8	Yes *(82 days)*	No	tacrolimus (8 µmol/l)[d] prednisone (10 mg qd) MMF[e] (750 mg bid)
7	68	M	Kidney *01.12.2006*	Hypertension	VGC 450 mg qd 3 months	4.4	Syndrome (probable)	0	Yes *(42 days)*	Gastritis (definite) *(62 days)*	tacrolimus (7 µmol/l)[d] prednisone (5 mg qd) MMF[e] (250 mg bid)

[a] Interval of time between transplantation and start of valganciclovir treatment
[b] Immunosuppressive maintenance regimen when valganciclovir treatment was introduced
[c] VGC = valganciclovir
[d] trough concentration
[e] MMF = mycophenolate mofetil
[f] MPS = mycophenolic sodium
[g] AZA = azathioprine
[h] This patient received a shorter treatment because of serum creatinine increase (dehydration, diuretic and nephrotoxic drugs). The treatment was reintroduced 10 days after interruption.

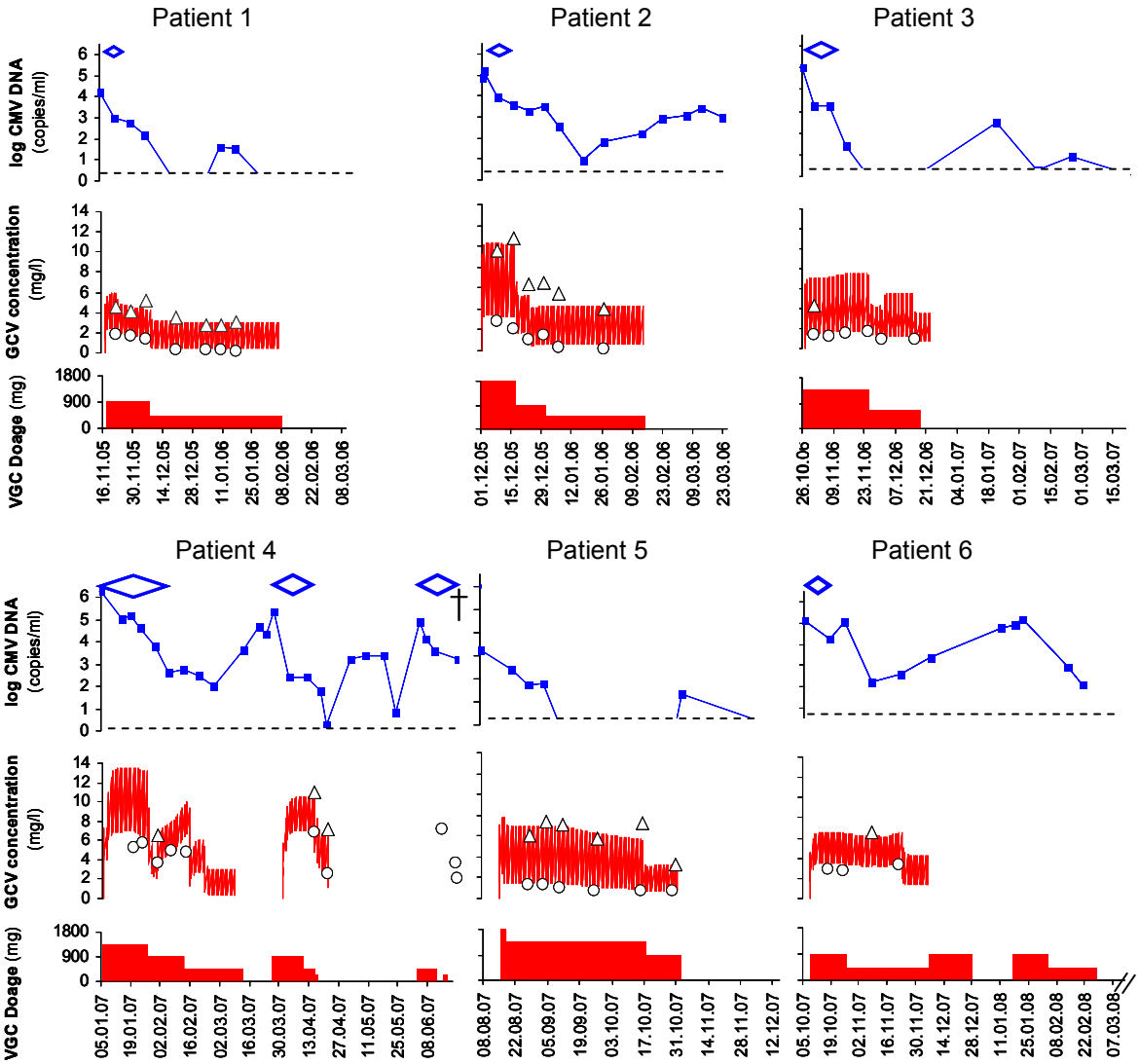

Figure 1: CMV treatment with valganciclovir in patients 1-6: valganciclovir dosage (red rectangle), ganciclovir plasma concentration (closed white circle: concentration measured at trough, closed white triangle: concentration measured 3h after last dose, red line: concentration predicted by population pharmacokinetic model), CMV viremia (blue square and solid blue line) and symptoms period (blue diamond)

Clinical symptoms resolved in all symptomatic patients with a median of 14.5 days of treatment (range: 5-28 days). The median viral load decline in these 7 patients was –1.2 log copies/ml after 1 week of treatment with a wide variation ranging from 0 to –1.8 log copies/ml. Viral clearance was obtained in five patients after a median time of 34 days (range: 28-82 days). In one patient, CMV DNAemia remained detectable but at a low level until lost of follow-up (48 days after 76 days of valganciclovir treatment discontinuation). Two SOT recipients (patients 4 and 7) developed 1-2 recurrent CMV disease episodes (follow-up: 9 months, range: 4-21 months) (table 1). Median baseline viral load was 5.0 log copies/ml (range: 3.2-5.1 log copies/ml) in 5 patients without recurrent episode and 3.9 and

6.3 log copies/ml in patients with relapse. Median ganciclovir C_{trough} during induction treatment was 1.5 mg/l (range: 0.8-3.3 mg/l) and C_{3h} 6.4 mg/l (range: 4.1-10.8 mg/l) in patients without relapse and 3.6 mg/l (range: 1.8-5.7 mg/l) and C_{3h} 6.5 mg/l (range: 5.3-6.7 mg/l) in patients with relapse, corresponding to ganciclovir exposure of 43.8 mg·h/l (32.7-74.3 mg·h/l) and 65.3 mg·h/l (44.3-117.9 mg·h/l), respectively. Overall, the median viral load decline of the 10 treatment courses in these 7 patients was –1.0 log/week with a wide variation ranging from 0 to –2.9 log/week.

Two patients had particularly complicate outcomes. Patient 4 was treated with oral valganciclovir a second time for a CMV syndrome 21 days after first therapy cessation and a third time for a CMV colitis 45 days after the second course discontinuation. CMV viremia decreased by –1.1 log copies/ml after 14 days, –3.0 log copies/ml after 15 days and -1.7 log copies/ml after 14 days during the first, second and third treatment, respectively (figure 1). Trough ganciclovir concentrations and AUC determined during these two episodes remained at the therapeutic range despite dose reduction while the patient renal function deteriorated. This patient developed hepatitis with moderate hepatocellular insufficiency during the second episode of CMV disease, possibly related to high ganciclovir plasma levels, requiring treatment interruption. Anti-CMV IgG measured after the first and the second course of valganciclovir showed a seroconversion after the second CMV disease episode only (14 months after transplantation). CMV specific T-cell response was not assessed. He developed however a third episode of CMV disease. His condition deteriorated due to cardiac decompensation and respiratory failure. He died from a septic shock and multi-organ failure while the CMV viral load was decreasing.

Patient 7 had an unusual course upon treatment initiation, his viral load first increasing by 1.2 log copies/ml after 16 days of treatment and then decreasing by -2.4 log copies/ml over the second week of treatment even though ganciclovir plasma levels remained stable. Median ganciclovir plasma levels and systemic exposure (AUC) during induction treatment were 1.9 mg/l (range 1.8-2.6 mg/l) and 46.5 mg·h/l (range: 44.3-47.5 mg·h/l, 4 measures), respectively, thus showing very little changes. Dose of mycophenolate mofetil was reduced (1.5 g per day to 0.5 g per day) 5 days before valganciclovir treatment initiation as leucopenia was detected and stopped one week after valganciclovir treatment initiation. Dose of prednisone was increase from 5 mg per day to 10 mg and tacrolimus plasma level were maintained at 8 µg/l. This patient developed a second episode of CMV disease 62 days after first treatment discontinuation. CMV viral load stabilized at 4 log copies/ml before valganciclovir treatment start and became undetectable while on treatment (figure 2). UL97 mutations were searched by gene sequencing but not detected. Anti-CMV IgM and IgG measured during the first and the second CMV episodes revealing low level of IgM from day

15 of first treatment course on while IgG remained undetectable until 4 months after the second treatment cessation (14.5 months after transplantation). CMV specific T-cell response assessed by ELISPOT assay after the first and second treatment (about 7 and 10.5 months after transplantation) showed low but rising numbers of IFN-γ-producing CMV-specific cells. Interestingly, this patient D+/R- for EBV developed also a high EBV viral load and did not build an EBV specific T cell response.

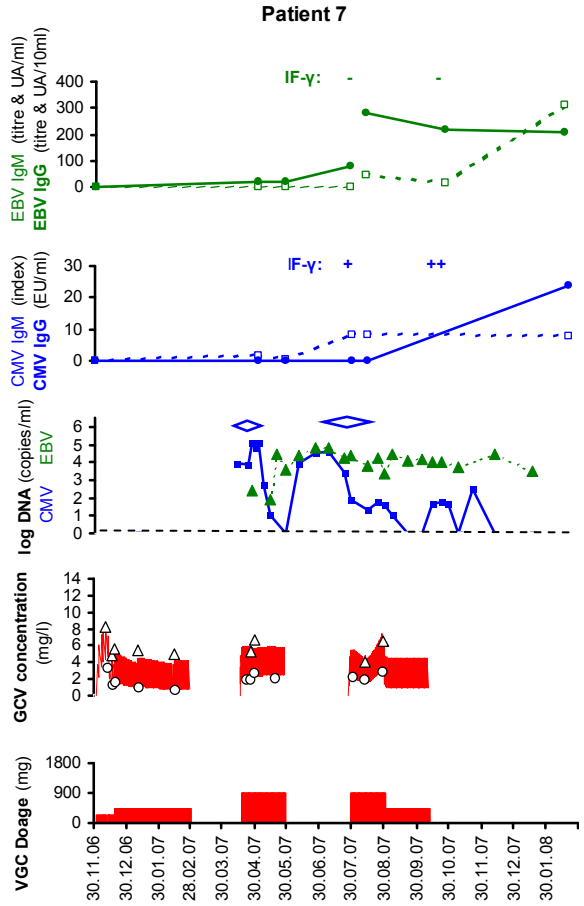

Figure 2: CMV treatment with valganciclovir in patient 7: valganciclovir dosage (red rectangle), ganciclovir plasma concentration (closed white circle: concentration measured at trough, closed white triangle: concentration measured 3h after last dose, red line: concentration predicted by population pharmacokinetic model), CMV viremia (blue square and solid blue line), symptoms period (blue diamond), EBV viremia (green triangle and dotted green line), anti-CMV IgM and IgG (IgM: closed white square and dotted blue line, IgG: blue circle and solid blue line), anti-EBV IgM and IgG (IgM: closed white square and dotted green line, IgG: closed green circle and solid green line), CMV specific T-cell response and EBV specific T-cell response (Interferon-γ, IF-γ -: negative; IF-γ +: positive)

5.1.5. Discussion and conclusion

We report on 7 recipients D+/R- treated with valganciclovir for CMV late disease after prophylaxis (n=6) and pre-emptively for CMV infection (n=1) with monitoring of CMV viral load and ganciclovir plasma level. This small number of observations reflects the single centre nature of our study and the rather good efficacy of prophylaxis (which was prescribed to all patients except to few liver transplant recipients), decreasing the incidence of CMV disease among SOT recipients.

We observed a widely variable response with delayed CMV viremia load decrease in 1/7 recipient and recurrent infection in 2/7 patients. The rate of CMV viral load decrease after 1 week of treatment was in the range of those reported with iv ganciclovir or oral valganciclovir [12,13], but viremia clearance was lower in our population including only D+/R- recipients. There was absolutely no indication that this variable response could be related to insufficient ganciclovir exposure, as shown by the result of ganciclovir plasma level monitoring revealing sufficient [14] or even rather higher concentrations in the patients with poor response. Ganciclovir needs to be bio-activated in infected cells into ganciclovir triphosphate to inhibit virus replication. In the absence of available clinical samples containing appropriate numbers of infected cells, ganciclovir plasma concentration is the only measurable surrogate of the actual active form concentration. Delayed viral clearance or recurrent infection seems thus more likely related to the absence of CMV cell-mediated immunity in a subset of patients experiencing primary infection while on immunosuppressive therapy [15]. However, the present study was not designed to assess the cell-mediated specific response to CMV. Emery et al., by comparing CMV replication dynamics in CMV-naïve and -experienced hosts, demonstrated that a higher drug efficacy was required to eliminate viral replication in non immune liver transplant recipients [16]. Additionally it has been shown that viral factors, such as infection with multiple CMV glycoprotein B genotypes, may also influence the response to antiviral therapy [17]. Interestingly, one patient experienced simultaneous primary infection by CMV and EBV, with delayed response to valganciclovir treatment and recurrent CMV infection. CMV primary infection has been reported to increase the risk of a EBV related post-transplant lymphoproliferative disease in high risk (D+/R-) EBV recipients [18]. Conversely, one wonders whether EBV infection could influence the course of CMV infection.

In conclusion, variable viral clearance could not be explained by a lower ganciclovir exposure in valganciclovir-treated patients, but was probably related to the immunological variability of seronegative recipients undergoing primary infection early after transplantation or after prophylaxis discontinuation or to viral factors.

5.1.6. References

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Disposition of valganciclovir during continuous renal replacement in two lung transplant recipients

N. Perrottet[1], C. Robatel[1], P. Meylan[2], M. Pascual[3], J.P. Venetz[3], J.D. Aubert[3], M.M. Berger[4], L.A. Decosterd[1], T. Buclin[1]

[1] Division of Clinical Pharmacology and Toxicology, University Hospital (CHUV), Lausanne, Switzerland

[2] Microbiology Institute, University Hospital (CHUV), Lausanne, Switzerland

[3] Organ Transplantation Centre, University Hospital (CHUV), Lausanne, Switzerland

[4] Dpt of Adult Intensive Care Medicine, University Hospital (CHUV), Lausanne, Switzerland

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Related appendices: 3.1, 3.2, 3.3 and 3.4

5.2. Disposition of valganciclovir during continuous renal replacement in two lung transplant recipients

5.2.1. Abstract

Objectives: To determine whether 450 mg every 48 h of valganciclovir for cytomegalovirus (CMV) prophylaxis provides appropriate ganciclovir exposure in solid organ transplant recipients during continuous renal replacement therapy (CRRT).

Patients and methods: Ganciclovir pharmacokinetics were intensively studied in two lung transplant recipients under valganciclovir 450 mg every 48 h over one dosing interval. *In vitro* experiments using blank whole blood spiked with ganciclovir further investigated exchanges between plasma and erythrocytes.

Results: Ganciclovir disposition was characterised by apparent total body clearance of 3.3 and 5.8 l/h, terminal half-life of 16.9 and 14.1 h, and apparent volume of distribution of 60.3 and 104.9 l in patients 1 and 2, respectively. The observed sieving coefficient was 1.05 and 0.96, and the haemofiltration clearance 3.3 and 3.1 l/h. *In vitro* experiments confirmed rapid efflux of ganciclovir from red blood cells into plasma, increasing the apparent efficacy of haemofiltration.

Conclusions: A valganciclovir dosage of 450 mg every 48 h appears adequate for patients under CRRT requiring a prophylaxis of CMV infection, providing concentration levels in the range reported under 900 mg once daily outside renal failure.

5.2.2. Introduction

Valganciclovir is currently supplanting oral ganciclovir in various indications, such as the prophylaxis of cytomegalovirus (CMV) infection in solid organ transplant patients. This prodrug is characterised by a better oral bioavailability. After administration, valganciclovir is hydrolysed to ganciclovir and extensively eliminated by the kidney, through both glomerular filtration and tubular secretion. Thus, in renal insufficiency, the dosage of valganciclovir has to be adjusted to the estimated glomerular filtration rate (GFR) [1]. The manufacturer suggests a scheme for dosage adaptation in function of GFR, but does not recommend the use of valganciclovir in patients requiring intermittent dialysis or continuous renal replacement therapy (CRRT). The high clearance of ganciclovir in healthy subjects (about

14 l/h) [1] indicates that tubular secretion contributes to a similar extent as glomerular filtration (7 l/h). CRRT replaces only GFR and usually does not exceed half of a normal GFR. Thus a rational dosage adjustment in CRRT patients would not exceed a quarter of the usual dose intensity, i.e. 450 mg every second day instead of 900 mg once daily. We aimed to confirm in two patients receiving CRRT through continuous veno-venous haemofiltration (CVVHF) that this dosing schedule provides adequate ganciclovir exposure in such a condition.

5.2.3. Patients and methods

This observational study was performed at the Lausanne University Hospital, Switzerland, with the approval of the local ethics committee, in two lung transplant recipients at risk for CMV infection.

Patient 1

A 49-year-old, 92 kg male patient was admitted for double lung transplantation in the context of idiopathic pulmonary fibrosis. A prophylaxis of valganciclovir was started on day 1 post-transplantation (900 mg once daily initially). On day 3, his condition deteriorated with cardiogenic and subsequent septic shock and multi-organ failure requiring initiation of CRRT. The CVVHF apparatus (Aquarius, Edwards Lifesciences S.A., St-Prex, Switzerland) was equipped with a 0.12 m^2 polyethersulfone fibre filter (Aquamax HF12, Edwards Lifesciences S.A.) connected through a double lumen venous catheter. On day 14, the treatment conditions were set to 300 ml/min blood flow, 3 l/h post-dilution flow (lactate-buffer electrolyte-glucose solution) and 150 ml/h patient's volume subtraction. The patient was anuric. He received his last valganciclovir 900 mg dose 48 h before switching to 450 mg dose every second day on the day of observation. Valganciclovir was given through nasogastric tube after dissolution of the tablet in warm water. Concomitant drug therapy consisted in norepinephrine, midazolam, fentanyl, sufentanil, heparin, mycophenolate, tacrolimus, methylprednisolone, vancomycin, voriconazole, tazobactam/piperacillin, insulin, esomeprazole, neostigmine. CRRT was followed by intermittent chronic haemodialysis. The patient died four months later, from septic shock.

Patient 2

A 54-year-old, 46 kg Asian male patient, was admitted for a second double lung transplantation because of bronchiolitis obliterans, *Mycobacterium, Pseudomonas* and *Aspergillus* infections of the first allograft. During the intervention haemorrhagic shock led to acute renal failure and the initiation of CRRT (same supplies as patient 1). A prophylaxis of valganciclovir was started on day 8 post-transplantation (450 mg every 48 h). (Valganciclovir tablet being crushed and dissolved in cold water before administration through nasogastric tube). On day 19, the CVVHF conditions were set to 250 ml/min blood flow, 1 l/h pre-dilution flow, 3 l/h post-dilution flow and 180 ml/h patient's volume subtraction. The patient was anuric. Concomitant drug therapy consisted in norepinephrine, propafenone, sufentanil, propofol, lorazepam, heparin, mycophenolate, cyclosporine, methylprednisolone, vancomycin, tazobactam/piperacillin, ethambutol, azithromycin, caspofungin, lamivudine, colistimethane, nystastin, insulin, esomeprazole. He died two months later from cardiac arrest.

Sample collection and assay

Blood samples were collected from both arterial and venous lines, along with filtrate samples from the output line, at times zero (before drug administration), 1, 2, 3, 4, 6, 12, 24, 36 and 48 h after administration of valganciclovir 450 mg. Blood samples were centrifuged and plasma and filtrate samples stored at –20°C until analysis.

Ganciclovir concentration in plasma was measured using validated high-performance liquid chromatography method after protein precipitation, on a reversed-phase C18 column, using a mobile phase gradient of sodium heptanosulfonate 0.4% buffer (pH 2.6) and acetonitrile, and spectrofluorimetric detection [2]. The lower limit of detection was 0.1 µg/ml, the inter-day coefficient of variation was lower than 3.5% and the range of inter-day deviations was comprised within –0.4 to +1.4%. Filtrate samples were analysed using calibration and quality controls prepared with blank filtrate.

Pharmacokinetic analysis

Ganciclovir concentration-time data in plasma and filtrate were analysed using non compartmental methods. The area under the curve during one dosing interval ($AUC_{0\text{-}48}$) was estimated by trapezoidal and log-trapezoidal methods and the apparent total body clearance (CL_{TOT}/F) was calculated as the dose divided by $AUC_{0\text{-}48}$. This analysis was completed by curve fitting with a two-exponential model, enabling to estimate an initial (λ_1) and a terminal

rate constant (λ_Z). The elimination half-life ($t_{1/2}$) was derived as $Log_e(2)/\lambda_Z$ and the apparent terminal volume of distribution (V_Z/F) as CL_{TOT}/λ_Z. Two approaches were used to determine the clearance of ganciclovir through the CRRT apparatus. The first is based on the amount of drug removed from blood, calculated from ganciclovir plasma prefilter (C_A) and postfilter (C_V) concentrations and defined as "haemofiltration clearance" (CL_{CRRT}). The second compares simultaneous filtrate and "arterial" plasma concentration. Their ratio is taken as sieving coefficient (S_C) and the filtration clearance (CL_F) is estimated by the product of S_C and total filtrate flow, reflecting the rate of drug appearance in the filtration fluid, divided by the circulating concentration. The recovery (R) in the filtrate is calculated as the ratio of CL_{CRRT} over CL_F. S_C, CL_{CRRT}, CL_F are calculated for each sampling time and averaged using the geometric mean [3].

5.2.4. Results and discussion

The concentration-time profiles of ganciclovir in patients 1 and 2 under 450 mg of valganciclovir every 48 h are shown in figure 1 and the resulting pharmacokinetic parameters are given in table 1. For patient 2, samples were collected over 24 h instead of 48 h because CRRT was interrupted after 24 h due to filter failure. C_{max}, C_{min} and AUC_{0-48} determined for patient 1 may be influenced by his prolonged dosage of 900 mg once a day, with a change to 900 mg every second day, two days before investigation. This may have affected AUC_{0-48} and CL_{TOT}/F but not CL_{CRRT}, S_C and CL_F.

Valganciclovir has proved successful for the prophylaxis of CMV in solid organ transplant recipients without renal impairment at 900 mg once daily [4]. The AUC calculated for patient 2 is in the range reported with 900 mg once daily (42-63 mg·h/l) [4-7] and patient 1 shows even a higher exposure. CL_{TOT}/F obtained in both patients are much lower than CL_{TOT}/F reported in the absence of renal impairment (10.2-15.5 l/h) [4-7] and correspond to values reported for patients with fairly reduced GFR [1]. Accordingly $t_{1/2}$ are longer than in patients without renal impairment (about 5 h in liver recipients [5] and 3.5 h in healthy subjects [1]), and are in the range reported for patients under CRRT receiving intravenous ganciclovir [8-10]. Vz/F determined in these two patients are in the range reported for healthy subjects and patients with and without renal impairment [1]. These results are in accordance with the almost exclusive renal elimination of ganciclovir, including a large component of tubular secretion which is not replaced by CRRT. In addition, the dose preparation and nasogastric tube administration seems not to grossly impair the drug absorption.

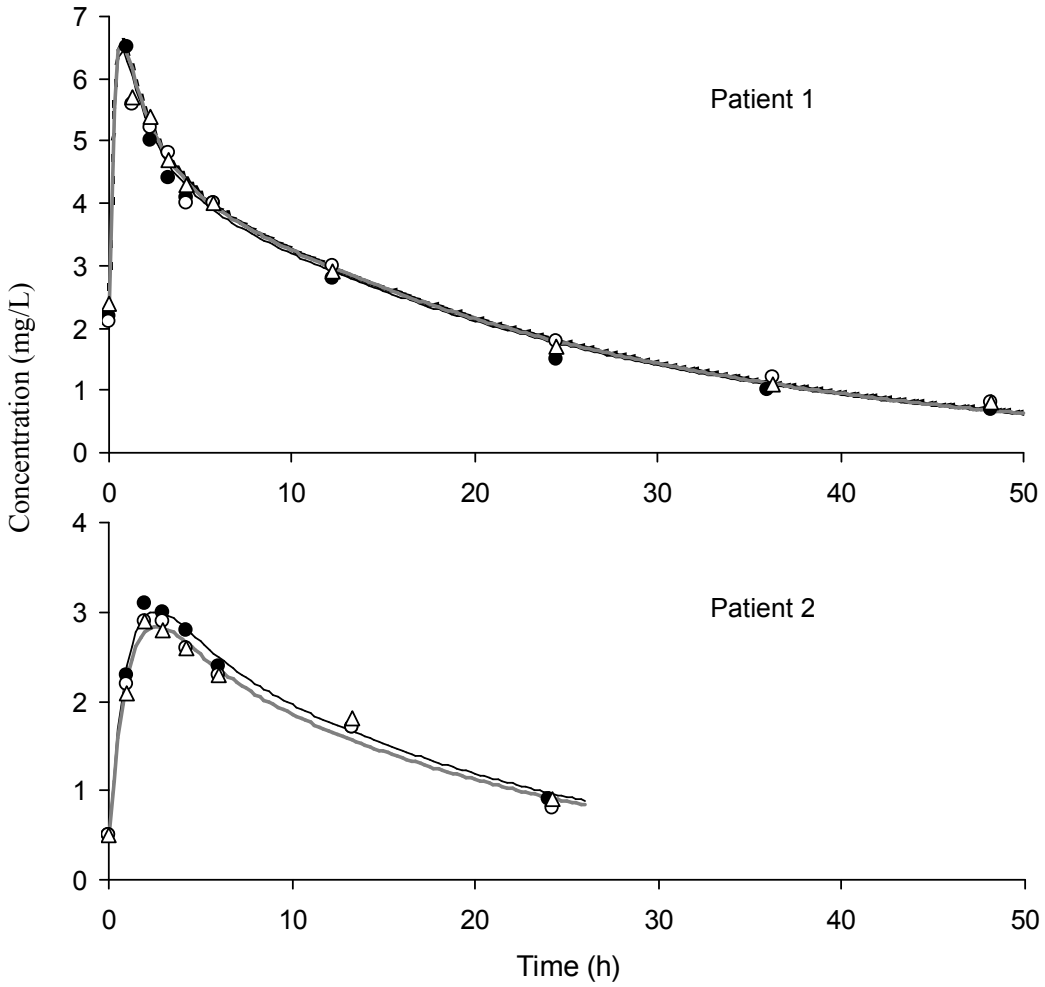

Figure 1: Concentration-time points and fitted biexponential curves of ganciclovir in "arterial" and "venous" lines and in filtrate in patients 1 (lower panel) and 2 (upper panel). (Closed circles and solid black line: "arterial" concentrations; open circles and dotted black line: "venous" concentrations; triangles and solid grey line: filtrate concentrations.)

Table 1: Pharmacokinetic Parameters

Patient	Dose (mg)	C_{max} (mg/L)	C_{min} (mg/L)	AUC_{0-48} (mgh/L)	λ_1 (h^{-1})	λ_Z (h^{-1})	$t_{1/2}$ (h)	CL_{TOT}/F (L/h)	V_Z/F (L)	CL_{CRRT} (L/h)	S_C	CL_F (L/h)	R (%)
1	450[a]	6.5	0.7	98.0	0.62	0.041	16.9	3.3	80.7	2.4	1.05	3.3	104
2	450[a]	3.1	0.2[b]	55.4	0.65	0.049	14.1	5.8	118.6	3.5	0.96	3.1	87

[a] corresponding to 324.1 mg of ganciclovir, [b] value extrapolated from λ_Z

In patient 1, CL_{CRRT} based on plasma disappearance rate and CL_F estimated from appearance in filtrate-dialysate accounted for approximately 70-100% of the apparent total clearance. Accordingly, the average recovery of ganciclovir was 104% and the sieving coefficient 1.05. In patient 2, CL_{CRRT} and CL_F accounted for approximately 50-60% of the apparent total clearance and indicated an average recovery of 87% and a sieving coefficient

of 0.96. These results are in accordance with sieving coefficients and percentage of total clearance achieved by CRRT previously reported for ganciclovir (0.75-95 and 40%-115% respectively) [8-10].

The profile (concentration *vs* time) of ganciclovir influx and efflux through red blood cell membranes is shown in figure 2. In the first phase (influx study), ganciclovir enters erythrocytes to reach an equilibrium between cells and plasma (erythrocytes/plasma ratio = 0.77). In the second phase (efflux study), ganciclovir leaves the erythrocytes following the concentration gradient between cells and plasma for attaining the same ratio.

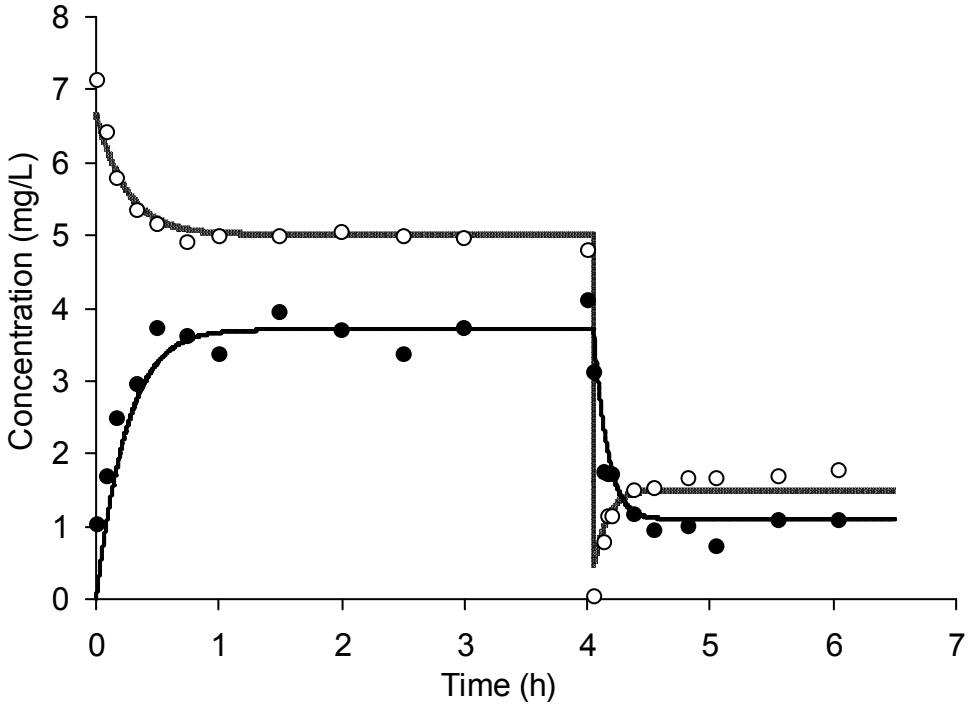

Figure 1: Concentration-time points and fitted exponential curves obtained from the in vitro ganciclovir permeation experiment. A 90 ml citrated blood volume obtained from a healthy donor was spiked with an amount of 500 µg ganciclovir (dissolved in 10 ml 0.9% NaCl) on time 0. During 4 h ("influx" phase), the blood pool was regularly sampled and ganciclovir concentration were followed up in the plasma (closed white circles and grey line) and in the red blood cells (closed dark circles and black line). On time 4 h, all the remaining plasma was separated by centrifugation and replaced with an identical volume of blank plasma from the same donor. Ganciclovir concentrations were again followed up in plasma and in erythrocytes during the next 2 h ("efflux" phase). The profile was fitted according to a two compartmental model with passive bidirectional diffusion (see text).

Of note, ganciclovir concentrations in plasma and red blood cell control samples remained stable for 4 and 6 h at 37°C and without any change of pH. Ganciclovir was previously reported to enter erythrocytes through nucleobase and nucleoside transporters [11]. In our experiment, we show that ganciclovir also leaves red blood cells in accordance to the concentration gradient. This phenomenon could play a role during blood filtration by CRRT

as the plasma/erythrocytes equilibrium is modified while ganciclovir is removed from plasma. Such exchanges occur in the haemofiltration filter and during post-dilution, providing an explanation for sieving coefficients and recovery fraction greater than the unity, as observed in patient 1. Thus, drug transport by red blood cells may increase CRRT efficacy for substances with significant distribution in the erythrocytes, a compartment often neglected in pharmacokinetics [12].

In conclusion, a 450 mg valganciclovir dose administered every 48 h achieves, in transplant recipients under CRRT, exposure levels similar to those observed under the usual dosage (900 mg once daily) recommended in the absence of renal failure.

5.2.5. References

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Chapter 6 : Conclusion & Perspectives

Presentation of chapter 6

What are the reproducibility and predictability of pharmacokinetic and pharmacodynamic profiles of valganciclovir? Which advice should be given to physicians in charge of transplant patients? In which indications should therapeutic drug monitoring be recommended?

The following chapter summarises our global conclusions. It highlights the results obtained during the clinical study, and the answers brought to the questions addressed in this research. The potential usefulness of ganciclovir therapeutic drug monitoring in organ transplant patients management is discussed.

The perspectives of this work are outlined at the end of the chapter 6.

6. Conclusion and perspectives

6.1. Conclusion

Valganciclovir pharmacokinetic and pharmacodynamic profiles were assessed in solid organ transplant recipients under valganciclovir prophylaxis or treatment for CMV infection by an observational study and a review of the current knowledge in the medical literature.

The systematic review revealed valganciclovir pharmacokinetic parameters fairly predictable in solid organ transplant recipients knowing patient's glomerular filtration rate and body weight. Unexplained interpatient variability was limited suggesting no major effect of undefined parameters. Bioavailability appeared reproducible and no problem of absorption was reported in solid organ transplant patients. However, no data were available in lung transplant, severe malabsorption and cystic fibrosis patients. In contrast valganciclovir and ganciclovir pharmacodynamic properties were too loose to define formal therapeutic or toxic intervals. Nevertheless, *in vitro* activity was reproducible against sensitive viral strains with IC_{50} close to 1 mg/l. Considering classical questions addressing the potential usefulness of drug TDM, the routine use of valganciclovir TDM in solid organ transplant recipients appeared not indicated. However, certain populations of patients are still poorly covered by current knowledge (e.g. patients with lung transplant, cystic fibrosis, severe malabsorption, unstable or rapidly changing renal function), leaving room for TDM usefulness in certain specific situations.

Our prospective observational population pharmacokinetic study in solid organ transplant patients under oral valganciclovir confirmed this evaluation with new original data. Systemic clearance was markedly influenced by the glomerular filtration rate (GFR), patient gender, and graft type (clearance/GFR = 1.7 in kidney, 0.9 in heart, and 1.2 in lung and liver recipients) with interpatient and interoccasion variabilities of 26 and 12%, respectively. Body weight and sex influenced central volume of distribution (V_1 = 0.34 l/kg in males and 0.27 l/kg in females [20% interpatient variability]). No significant drug interaction was detected with immunomodulator agents or substrates of organic anion transporters. VGC prophylactic efficacy and tolerability were good (table 1), without detectable dependence on GCV profile. This high level of response confirms the adequacy of the dosage used in our population of patients but also impedes the possibility of showing clear concentration-effect relationships. It also explains the small proportion of recipients treated with valganciclovir for overt CMV disease. Late CMV disease cases were restricted to D+/R- recipients, which are known to be at higher risk related to absence of pre-existing anti-CMV immunity. Delayed viral load

response observed in 1/7 recipient and recurrent CMV infection in 2/6 were not related to insufficient ganciclovir exposure.

Table1: Description of the study prophylactic arm and the study patients (mean ± SD and absolute value).

Recipient	**N**	**GFR** (ml/min)	**Dose/day** (mg)	**CMV DNA detection during prophylaxis**	**CMV disease during prophylaxis**
KIDNEY					
D+/R-	9	52 ± 18	420 ± 80	0	0
R+	25	60 ± 19	440 ± 50	0	0
LUNG					
D+/R-	3	127 ± 19	750 ± 220	1	0
R+	4	93 ± 20	900	0	0
HEART					
D+/R-	2	85 ± 34	515 ± 170	1	0
R+	4	78 ± 13	515 ± 170	1	0
All	**49**			**3**	**0**

In conclusion, pharmacokinetic characteristics of valganciclovir are fairly predictable, while its pharmacodynamic profile is loosely defined. This work highlights the importance of thorough adjustment of VGC dosage to renal function and body weight. Considering the good predictability and reproducibility of GCV profile after oral VGC in solid organ transplant recipients, routine TDM does not appear to be clinically indicated. However, GCV plasma measurement may still bring clinically helpful information in specific situations.

6.2. Perspectives

Pharmacokinetic studies would be needed in subgroups of patients poorly evaluated to date such as patients with lung transplant, cystic fibrosis, severe malabsorption, or unstable or rapidly changing renal function. A limitation of current studies is the small number of such patients observed. The creation of the Swiss Cohort of Transplant Recipients could be taken as an opportunity to go beyond this limitation and extend observations to defined subgroups of patients, in whom questions remain upon the absorption and disposition of valganciclovir.

Valganciclovir is a very recently introduced drug, and every aspects of its clinical use have probably not been entirely covered. The availability of ganciclovir TDM facilities in a reference center may enable further research to optimise the therapeutic and prophylactic use of valganciclovir, and to document exposure and guide clinical decision in selected problematic cases. In that respect, it is of importance to not only rely on a validated analytical method, but also provide scientific elements for a rational interpretation of concentration results. Population pharmacokinetic studies should therefore ideally be combined with pharmacodynamic assessments.

For example, therapeutic efficacy of valganciclovir treatment appeared suboptimal in D+/R- recipients despite adequate ganciclovir exposure, enhancing the key role of immune response. The small number of such patients could however not lead to significant conclusions. Monitoring of the response to valganciclovir treatment in R+ comparing to D+/R- recipients with measurement of ganciclovir plasma concentration could be used to evaluated the treatment efficacy difference between serostatus patterns as well as within each group. More sophisticated pharmacokinetic-pharmacodynamic modelling could help to delineate the relative contribution of immunological and pharmacological factors in CMV infection management.

The concentration-effect relationships that we have studied here appear too loose to define formal therapeutic or toxic intervals. One explanation for this problem is that ganciclovir is activated after intracellular entry into phosphorylated derivatives, which selectively accumulate in infected cells. Ganciclovir efficacy may thus be better correlated to intracellular ganciclovir triphosphate concentrations, which would possibly represent a better marker for actual exposure to the active agent. Nevertheless, such intracellular monitoring would require the development of highly elaborate analytical methods with extensively low quantification threshold, to be applied on fractions of peripheral blood mononuclear cells or other suitable cell subpopulations. Such development could represent further opportunities for pharmacogenetic explorations. In fact, the different steps characterizing valganciclovir mechanism of action may each be affected by polymorphisms. Absorption through PEPT transporters, hydrolysis into ganciclovir, renal elimination through anionic tubular transport, penetration into cells, bioactivation through phosphorylation.

Large number of patients would however be required for such pharmacogenetic explorations. We feel it suitable that further developments of the clinical research on valganciclovir keep the patients' welfare as main objective.

Appendices

Protocole de dosage de l'aciclovir et du ganciclovir

Références :

1. Loregian A, Gatti R, Palu G, De Palo EF. Separation methods for acyclovir and related antiviral compounds. J of Chromatography B 764, 289-311 (2001).

Produits chimiques :

-	Aciclovir	Zovirax 250 mg ref : 944511(CHUV)
-	Ganciclovir	Cymevene 500 mg ref: 1347817(CHUV)
-	9-Ethyl- guanine	Sigma ref: E-4267
-	Heptanosulfonate de Sodium	Merck ref: 1.18306.
-	Acetonitrile	Backer ref: 8143
-	TCA 20%	Merck ref : 1.09415
-	Acide acétique glacial 100%	Merck
-	Eau Ultrapure MilliQ	

Equipements :

- Tubes Eppendorf 1.5 - 2.0 ml + portoirs
- Pipettes Gilson, P1000 et P200 et les embouts correspondants
- Vortex
- Centrifugeuse Hettich universal 16R pour tubes Eppendorf
- Vials en verre pour HPLC (Agilent)
- Caps pour vials HPLC (Agilent)
- Inserts pour vials HPLC (Agilent)
- HPLC Agilent 1050 avec détecteur spectrofluorimétique LC240 Perkin-Elmer (localisé au BH18-218) (aciclovir-ganciclovir)
- Colonne HPLC Macherey-Nagel Nucleosil 100-5-**C18**, 5 μm, 250 x 4mm + pré-colonne Macherey-Nagel Nucleosil 100-5-**C18** 8x4mm

Normes de sécurité :

Les manipulations avec du matériel biologique infectieux (plasma CMV+) se font toujours avec des gants de protection.

Les manipulations avec le sang de patient CMV+ (récolte du plasma après centrifugation) se font sous une hotte d'aspiration avec des gants et si nécessaire (absence de protection vitrée) en portant des lunettes de protection. Le processing du plasma se fait sous une hotte d'aspiration avec des gants jusqu'à l'étape de précipitation des protéines avec le TCA 20%. Les tips utilisés pour pipeter le sang, le plasma des patients ainsi que le résidu après précipitation des protéines sont éliminés dans un bidon fermé, réservé à cet effet (identifié CMV, localisé au labo BH218-224). Ces bidons sont acheminés régulièrement pour autoclavage selon la même procédure que pour le matériel HIV+.

Solutions :

- Solution mère mixte aciclovir, ganciclovir 1mg/ml :

  Aciclovir 25mg/ml : reconstituer 1 ampoule de Zovirax 250 mg avec 10.0 ml H_2O bidistillée

  Ganciclovir 50 mg/ml : reconstituer 1 ampoule de Cymevene 500 mg avec 10.0 ml H_2O bidistillée

  Solution mère mixte 1mg/ml : 2.0 ml de solution aciclovir 25mg/ml + 1.0 ml ganciclovir 50 mg/ml ad 50.0 ml H_2O bidistillée

- Solution avec étalon interne: 9-éthyl-guanine 5 μg/ml

  Diluer la solution stock 9-éthyl-guanine 100 μg/ml : 1/20 avec H_2O bidistillée

  Solution stock 9-éthyl-guanine 100 μg/ml : dissoudre (aux ultra-sons) 10 mg de 9-éthyl-guanine dans environ 50 ml H_2O bidistillée, avec 1 ml d'acide acétique et 10 ml de MeOH, puis compléter à 100.0 ml avec H_2O bidistillée.

- Solvant C : acétonitrile

- Solvant A : solution 4.00 g d'heptanosulfonate de sodium dans 1000 ml H_2O bidistillée ; + acide acétique 100% ad pH 2.60 (suffit pour environ 20 injections)

Calibrateurs et contrôles :

Droite de calibration : Il faut toujours préparer deux mesures par point pour générer la droite de calibration. La première série d'échantillons de calibration débutera la séquence et la deuxième la terminera. On fera ensuite la moyenne des deux pour calibrer la méthode. Cette droite comporte pour les échantillons dans le **plasma** 6 tubes (A à F) avec des concentrations de aciclovir/ganciclovir allant de 100 ng/ml à 10 μg/ml.

Contrôles : Ces échantillons sont utilisés comme contrôles de qualité (QC) et permettent de vérifier la présence d'une dérive des valeurs.

Solutions mixtes pour calibrateurs dans le plasma : diluer la solution stock = **aciclovir, ganciclovir 1 mg/ml**.

TUBES	Solution mixte aciclovir ganciclovir **1mg/ml** / H2O	Volume Dilution (ml)	Volume de Plasma ajouté (ml)	Conc. aciclovir ganciclovir (ng/ml)
A	1/10	2.0	18	10 000
B	1/20	2.0	18	5000
C	1/100	2.0	18	1000
D	1/200	2.0	18	500
E	1/400	2.0	18	250
F	1/1000	2.0	18	100

Solution mixte **aciclovir,ganciclovir 100 µg/ml** : diluer la solution stock aciclovir, ganciclovir 1 mg/ml 1/10 avec H_2O bidistillée.

TUBES	Solution mixte aciclovir ganciclovir **100 µg/ml** / H2O	Volume Dilution (ml)	Volume de Plasma ajouté (ml)	Conc. aciclovir ganciclovir (ng/ml)
G	8/10	2.0	18	8 000
H	3/10	2.0	18	3 000
I	7.5/100	2.0	18	750

Vortexer les solutions quelques minutes puis aliquoter dans des tubes en plastique 2.0 ml à raison de 1.2 ml. Stocker les tubes au congélateur à -20°C.

Mode opératoire :

Documenter les analyses sur la cahier ciclovir Cl - A (Analyse) :

- Date/ nom de l'opérateur/ échantillons analysés et nom de l'étude.
- Le nom de la séquence et de la méthode de calibration HPLC auront pour nom : CIA - numéro de page du cahier.

Préparation des solutions :

- Solvant A
- Solution diluée de 9-éthyl-guanine 5 µg/ml

Préparation des échantillons :

- Prendre une série d'échantillons pour la droite de calibration (6 tubes), une série de contrôles (3 tubes) et une série d'échantillons de patients du congélateur et laisser revenir à RT.
- Vortexer les échantillons et préparer une série d'Eppendorfs (2.0 ml) avec dans chacun 1.0 ml d'échantillon plasma, ajouter 250 µl d'étalon interne (9-éthyl-guanine 5 µg/ml).
- Vortexer, ajouter 250 µl de TCA 20%.
- Vortexer et centrifuger 10 min. à 4°C à 14'000 t/min.
- Reprendre le surnageant (1.0 ml) dans tubes et évaporer sous azote à 37°C (environ 2 à 3 heures) (hotte flux laminaire BH18-224)
- Reprendre le résidu dans **100 µl** de **phase mobile A**
- Vortexer 1 fois, laisser au repos 5-10 min puis vortexer encore 1 fois.
- Transférer les 100 µl dans des Eppendorfs (1.5 ml) et centrifuger 10 min. à 4°C à 14'000 t/min.
- Transférer le surnageant dans les vials HPLC bruns correspondants. Fermer les vials et les positionner sur les racks de l'HPLC.

Analyse par HPLC :

Edition de la méthode CICLO11 (CICLO11.M)

Solutions : A : 0.4% heptanesulfonate de sodium, ad pH 2.60 avec acide acétique
C : MeCN

T°C : colonne C18 250 mm à température ambiante

Volume d'injection : **30 µl**

Flow : 1.0 ml/min.

Stop time : 42.00 min.

Gradient :

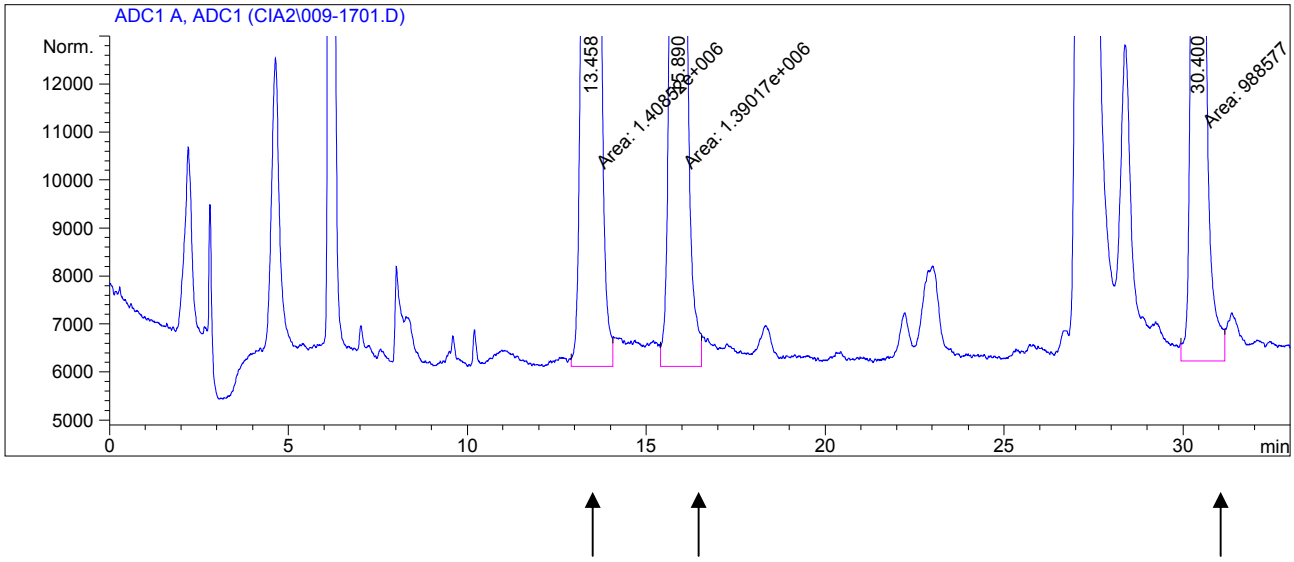
Temps (min.)	Sol. A Tampon	Sol. C MeCN
0.00	100 %	0 %
19.00	93%	7%
31.00	86%	14%
31.01	0%	100 %
36.00	0 %	100 %
36.01	100 %	0%
42.00	100 %	0%

Aciclovir-ganciclovir
Détection spectrofluorimétique LC240 Perkin-Elmer : excitation : 260 nm
émission : 380 nm

Facteur d'atténuation : 256
Facteur de réponse : 4

Attention : n'oubliez pas 58 CTRL pour fonctionnement continu.

Profil chromatographique typique, ci après (feuille jointe):



Ganciclovir Aciclovir Etalon Interne

Préparation de l'HPLC :

Enclencher les appareils

- Imprimante
- Ecran puis le disque dur, ne pas entrer dans le réseau Novell
- HPLC : tous les éléments
- Ouvrir « HP ChemStation » puis « Instrument online »

Travail sur « ONLINE » :

- * Extraire la méthode : *Method / Load method / CICLO11.M* (méthode de dosage *non calibrée*)
  Purger les lignes (ligne A : solution A et ligne C : MeCN). Laisser rincer jusqu'à ce que la ligne de base soit stable avec MeCN 100% puis avec MeCN/Solution A 50/50 puis avec Solution A 100% (->ligne de base et pression stables).
- Identifier les échantillons : *Sequence / Sequence table*. (possibilité de reprendre la table d'une séquence précédente et de changer les données : *File/ Load sequence* puis *Sequence / Sequence Table*) Remplir le tableau qui apparaît ainsi :
  - line : n° de l'injection
  - vial : n° de l'échantillon
  - sample name : nom de l'échantillon en référence au cahier (ex. CI-A-7 P 0025)
  - méthode name : choisir la méthode qui correspond à l'analyse et taper « enter » (CICLO11)
  - injection/éch. : en général on fait 1 injection par échantillon; inscrire 2 si on veut deux injections dans le même vial.
  - injVolume : volume d'échantillon injecté, ne rien mentionner, le volume est déjà sélectionné dans les paramètres de la méthode.
  - Introduire à la dernière ligne la méthode de rinçage : CYCLRINC mais ne pas mettre de numéro de vial mais mettre dans la colonne injection : 1.

  Ne pas s'occuper des autres cases non traitées ci-dessus.
- Sauver la séquence : *Sequence / Save as :* inscrire le nº du cahier (CIA7, p.ex., comme pour la méthode)
- Définir où les résultats vont être stockés : Sequence / Sequence parameter
  - Operator name : inscrire son nom
  - First-file name XXX.XXXX.D
    Subdirectory : inscrire le nº du cahier (CIA7, p.ex., comme pour la méthode et la séquence)
  - Shutdown Mettre une croix devant dans la case : *post - sequence - cmd/macro*
    Afficher : macro « shutdown », go en cliquant sur la flèche (case en dessous de post-sequ.)
    NRDY Time out : 00 min
- Sauver à nouveau la séquence : Sequence / Save
- Imprimer la séquence : *Sequence/Print sequence/* mettre une croix dans les cases : *Sequence Parameters* et *Sample (quantification part) log* table. Cliquer ensuite sur *Print*
- Positionner les vials dans les racks en commençant par la première série d'échantillons de calibration (la plus faible concentration en première position) et terminer avec la deuxième série d'échantillons de calibration (la plus haute concentration en dernière position). Insérer un échantillon de contrôle tous les 4 échantillons de patient.
- Vérifier que la pression soit comprise entre 140 et 170 bars
- Pour faire un Run d'essai, *Run Control / Run Method* et choisir l'échantillon (n° de vial) qui doit être injecté (de préférence injecter un ancien contrôle).
- Lancer la séquence : *Sequence table / Run sequence* ou *Run Control Run Sequence*
- Lorsque l'analyse est terminée il faut toujours rincer le système à l'aide de la méthode CYCLRINC normalement déjà programmée à la fin de la séquence, le cas échéant, *Method / Load Method / CYCLRINC*.m afin d'éliminer les restants de tampon qui peuvent précipiter dans les capillaires.

Recommencer au point -*, si on relance une nouvelle analyse, sinon éteindre les appareils : *Instrument / Système « OFF ». File / Exit.* Fermer l'hélium, éteindre l'HPLC.

Courbe de calibration et résultats :

Travail sur « OFFLINE »

A) Travailler un chromatogramme :

- Afficher les données : *View / Data Analysis*
- Extraire la méthode et la séquence : *File / Load method* : nº de la méthode (ex. CICLO11)
  File / Load sequence : nº de la séquence (ex. CIA7)
- Extraire le dossier : *File / Load signal* : Sortir le dossier qui contient les chromatogrammes
- Ouvrir un des fichiers de mesure (nº que lui a donné l'HPLC, ex : 001.0101.D, n°vial puis n°injection). Le chromatogramme s'affiche.

  (Changer, si nécessaire, la graduation au niveau de l'écran : *Graphic / Signal preferences* : changer les paramètres, *OK*. Modifier, si nécessaire, la base du pic : *Integration / Tangent skim* : cliquer, glisser, double-cliquer
  Couper , si nécessaire, un pic : *Integration / draw base* : cliquer, glisser, double-cliquer. *Integration / split pic* : double cliquer à l'endroit où l'on veut couper le pic.)

B) Etablir une courbe de calibration :

- 1ère valeur du 1er point : *File / Load signal* : nº du fichier. *Calibration /* : cliquer dans la case *new table*, « Enter ».La table de calibration apparaît; compléter la table : identifier le pic du ganciclovir et de l'aciclovir et indiquer la quantité injectée (calculée à partir des concentrations standards), identifier ensuite le pic de l'Etalon Interne (9-ethyl-guanine) et la quantité injectée (5000 ng/ml). Mettre une croix sous la case *Ref.*
  Seulement pour l'Etalon Interne : mettre une croix sous la case *Ref* et sous *ISTD* (Standard interne), écrire 1 sous la case « # » (3ème pic qui apparaît en fonction du temps). Ceci indique qu'il s'agit du pic de référence.
- Sauver la méthode : *File / **Save as** / Method* : nº de la méthode + c (ex. CIA7**C**.m). La méthode de calibration est sauvée, à l'ajout de chaque nouveau point de la droite de calibration à nouveau sauver la méthode : *File / **Save**/ Method*
- 1ère valeur du 2ème point : *File / Load signal* : nº du fichier. *Calibration/* : cliquer dans la case : *Add level* et inscrire: 2 dans la case *Level* « Enter ». La table apparaît : compléter la quantité d'Etalon Interne (toujours la même) et la quantité de ganciclovir et d'aciclovir (calculée à partir des concentrations standards). Sauver la méthode : *File / **Save**/ Method*
- Continuer de même avec tous les autres points de la calibration en vérifiant toujours le niveau de la calibration. Sauver la méthode : *File / **Save**/ Method* à chaque fois.
- 2ème valeur du 1er point (même concentration que la première) . *File / Load signal* : nº du fichier. *Calibration/recalib* : cliquer dans la case *Average*. Vérifier que le niveau de calibration (case: level) soit le même que pour la 1ère valeur càd 1), « Enter ». La table de calibration apparaît, la moyenne des deux premiers points est déjà faite, cliquer « OK ». Sauver la méthode : *File / **Save**/ Method.*
- 2ème valeur du 2ème point : (même concentration que la première) . *File / Load signal* : nº du fichier. *Calibration/recalib* : cliquer dans la case *Average*. Vérifier que le niveau de calibration (case: level) soit le même que pour la 1ère valeur càd 2), « Enter ». La table de calibration apparaît, la moyenne des deux premiers points est déjà faite, cliquer « OK ». Sauver la méthode : *File / **Save**/ Method.*
- Continuer de même avec tous les autres points de la calibration en vérifiant toujours le niveau de la calibration. Sauver la méthode : *File / **Save**/ Method*
- Lorsque tous les points sont introduits, vérifier les paramètres suivants :*Calibration / Calibration settings* : Amount : ng, Curve : linear, origine : ignore, weight : linear (Amnt), - *Report / Specify report* : calculate ISTD.
- Imprimer la droite de calibration et la table : *Calibration / Calibration curve* : Print et *Calibration / Calibration table :* print.
- Vérifier que la légende de l'axe des X est : AMOUNT RATIO et celle de l'axe des Y est : AREA RATIO.
- Sauver la méthode : *File / **Save**/ Method*, cela indique que la méthode est maintenant calibrée, et prête pour l'analyse quantitative de la série d'échantillons.
- Pour calculer la quantité de ganciclovir et d'aciclovir dans les contrôles et les échantillons : *File / Load signal*s : nº du fichier. *Report /Specify report* cocher la case Print. *Report /Print Report*. L'ordinateur imprime le chromatogramme de l'échantillon et la quantité de ganciclovir et d'aciclovir /injection qu'il a calculé à partir de l'équation de la droite de calibration. Ces valeurs sont données en ng /inj.
- Aller sur M/PCL/Commun/Labo/Ciclovir-statistiques/aciclovir-gancilovir_2005 et compléter les valeurs de QC de contrôles, les pentes de la droite et ordonnées à l'origine et r^2 pour le ganciclovir et d'aciclovir.

Protocole de dosage de l'aciclovir et du ganciclovir pour la NEONATOLOGIE (CICLNEO)

Références :

1. Loregian A, Gatti R, Palu G, De Palo EF. Separation methods for acyclovir and related antiviral compounds. J of Chromatography B 764, 289-311 (2001).

Produits chimiques :

- Aciclovir	Zovirax 250 mg ref : 944511(CHUV)
- Ganciclovir	Cymevene 500 mg ref: 1347817(CHUV)
- 9-Ethyl- guanine	Sigma ref: E-4267
- Heptanosulfonate de Sodium	Merck ref: 1.18306.
- Acetonitrile	Backer ref: 8143
- TCA 20%	Merck ref : 1.09415
- Acide acétique glacial 100%	Merck
- Eau Ultrapure MilliQ	

Equipements :

- Tubes Eppendorf 1.5 + portoirs
- Pipettes Gilson, P1000 et P200 et les embouts correspondants
- Vortex
- Centrifugeuse Hettich universal 16R pour tubes Eppendorf
- Vials en verre pour HPLC (Agilent)
- Caps pour vials HPLC (Agilent)
- Inserts pour vials HPLC (Agilent)
- HPLC Agilent 1050 avec détecteur spectrofluorimétique LC240 Perkin-Elmer (localisé au BH18-218) (aciclovir-ganciclovir)
- Colonne HPLC Macherey-Nagel Nucleosil 100-5-**C18**, 5 μm, 250 x 4mm + pré-colonne Macherey-Nagel Nucleosil 100-5-**C18** 8x4mm

Normes de sécurité :

Les manipulations avec du matériel biologique infectieux (plasma CMV+) se font toujours avec des gants de protection.

Les manipulations avec le sang de patient CMV+ (récolte du plasma après centrifugation) se font sous une hotte d'aspiration avec des gants et si nécessaire (absence de protection vitrée) en portant des lunettes de protection. Le processing du plasma se fait sous une hotte d'aspiration avec des gants jusqu'à l'étape de précipitation des protéines avec le TCA 20%. Les tips utilisés pour pipeter le sang, le plasma des patients ainsi que le résidu après précipitation des protéines sont éliminés dans un bidon fermé, réservé à cet effet (identifié CMV, localisé au labo BH218-224). Ces bidons sont acheminés régulièrement pour autoclavage selon la même procédure que pour le matériel HIV+.

Solutions :

- Solution mère mixte aciclovir, ganciclovir 1mg/ml :

  Aciclovir 25mg/ml : reconstituer 1 ampoule de Zovirax 250 mg avec 10.0 ml H_2O bidistillée

  Ganciclovir 50 mg/ml : reconstituer 1 ampoule de Cymevene 500 mg avec 10.0 ml H_2O bidistillée

  Solution mère mixte 1mg/ml : 2.0 ml de solution aciclovir 25mg/ml + 1.0 ml ganciclovir 50 mg/ml ad 50.0 ml H_2O bidistillée

- Solution avec étalon interne: 9-éthyl-guanine 5 μg/ml

  Diluer la solution stock 9-éthyl-guanine 100 μg/ml : 1/20 avec H_2O bidistillée

  Solution stock 9-éthyl-guanine 100 μg/ml : dissoudre (aux ultra-sons) 10 mg de 9-éthyl-guanine dans environ 50 ml H_2O bidistillée, avec 1 ml d'acide acétique et 10 ml de MeOH, puis compléter à 100.0 ml avec H_2O bidistillée.

- Solvant C : acétonitrile

- Solvant A : solution 4.00 g d'heptanosulfonate de sodium dans 1000 ml H_2O bidistillée ; + acide acétique 100% ad pH 2.60 (suffit pour environ 20 injections)

Calibrateurs et contrôles :

Droite de calibration : Il faut dans ce cas précis préparer uniquement une mesure par point pour générer la droite de calibration. Ceci en raison de la quantité insuffisante d'extrait pour permettre deux injections par niveau de calibration. La série d'échantillons de calibration débutera la séquence. Cette droite comporte pour les échantillons dans le **plasma** 6 tubes (A à F) avec des concentrations de aciclovir/ganciclovir allant de 100 ng/ml à 10 μg/ml.

Contrôles : Ces échantillons sont utilisés comme contrôles de qualité (QC) et permettent de vérifier la présence d'une dérive des valeurs.

Echantillons patient:

Un volume minimal de 100 μl de plasma est nécessaire pour faire cette analyse. Le prélèvement doit donc se faire dans une Microvette® 300 (= 0.3 ml serum, bouchon blanc). Après centrifugation 5-10 min à 1000 rpm à +4° C (Beckmann Centrifuge, Model J6B), transférer 100 μl de plasma directement dans un Eppendorf de 1.5 ml.

Solutions mixtes pour calibrateurs dans le plasma : diluer la solution stock = **aciclovir, ganciclovir 1 mg/ml**.

TUBES	Solution mixte aciclovir ganciclovir **1mg/ml** / H2O	Volume Dilution (ml)	Volume de Plasma ajouté (ml)	Conc. aciclovir ganciclovir (ng/ml)
A	1/10	2.0	18	10 000
B	1/20	2.0	18	5000
C	1/100	2.0	18	1000
D	1/200	2.0	18	500
E	1/400	2.0	18	250
F	1/1000	2.0	18	100

Solution mixte **aciclovir,ganciclovir 100 µg/ml** : diluer la solution stock aciclovir, ganciclovir 1 mg/ml 1/10 avec H_2O bidistillée.

TUBES	Solution mixte aciclovir ganciclovir **100 µg/ml** / H2O	Volume Dilution (ml)	Volume de Plasma ajouté (ml)	Conc. aciclovir ganciclovir (ng/ml)
G	8/10	2.0	18	8 000
H	3/10	2.0	18	3 000
I	7.5/100	2.0	18	750

Vortexer les solutions quelques minutes puis aliquoter dans des tubes en plastique 2.0 ml à raison de 1.2 ml. Stocker les tubes au congélateur à -20°C.

Mode opératoire :

Documenter les analyses sur la cahier ciclovir CI - A (Analyse) :

- Date/ nom de l'opérateur/ échantillons analysés et nom de l'étude.
- Le nom de la séquence et de la méthode de calibration HPLC auront pour nom : CIA - numéro de page du cahier.

Préparation des solutions :

- Solvant A
- Solution diluée de 9-éthyl-guanine 5 µg/ml

Préparation des échantillons :

- Prendre une série d'échantillons pour la droite de calibration (6 tubes), une série de contrôles (3 tubes) et une série d'échantillons de patients du congélateur et laisser revenir à RT.
- Vortexer les échantillons et préparer une série d'Eppendorfs (1.5 ml) avec dans chacun 0.1 ml d'échantillon plasma, ajouter 25 µl d'étalon interne (9-éthyl-guanine 5 µg/ml).
- Vortexer, ajouter 25 µl de TCA 20%.
- Vortexer et centrifuger 10 min. à 4°C à 14'000 t/min.
- Reprendre le surnageant (0.1 ml) dans tubes et évaporer sous azote à 37°C (environ 2 à 3 heures) (hotte flux laminaire BH18-224)
- Reprendre le résidu dans **50 µl** de **phase mobile A**
- Vortexer 1 fois, laisser au repos 5-10 min.
- Transférer les 50 µl dans des Eppendorfs (1.5 ml) et centrifuger 10 min. à 4°C à 14'000 t/min.
- Transférer le surnageant dans les vials HPLC bruns correspondants. Fermer les vials et les positionner sur les racks de l'HPLC.

Analyse par HPLC :

Edition de la méthode CICLNEO (CICLNEO.M)

Solutions : A : 0.4% heptanesulfonate de sodium, ad pH 2.60 avec acide acétique
C : MeCN

T°C : colonne C18 250 mm à température ambiante

Volume d'injection : **30 µl Attention ! descendre l'aiguille: - 2 mm** (draw position)

Flow : 1.0 ml/min.

Stop time : 42.00 min.

Gradient :

Temps (min.)	Sol. A Tampon	Sol. C MeCN
0.00	100 %	0 %
19.00	93%	7%
31.00	86%	14%
31.01	0%	100 %
36.00	0 %	100 %
36.01	100 %	0%
42.00	100 %	0%

Aciclovir-ganciclovir
Détection spectrofluorimétique LC240 Perkin-Elmer : excitation : 260 nm
émission : 380 nm
Facteur d'atténuation : 256
Facteur de réponse : 4

Attention : n'oubliez pas 58 CTRL pour fonctionnement continu.

Préparation de l'HPLC :

Enclencher les appareils

- Imprimante
- Ecran puis le disque dur, ne pas entrer dans le réseau Novell
- HPLC : tous les éléments
- Ouvrir « HP ChemStation » puis « Instrument online »

CENTRE HOSPITALIER UNIVERSITAIRE VAUDOIS

DEPARTEMENTS DE MEDECINE ET DE CHIRURGIE

Institut de Microbiologie
Pr A. Telenti, Médecin chef
E-mail : Amalio.Telenti@chuv.hospvd.ch

Division de Pharmacologie et Toxicologie Cliniques
Pr J. Biollaz, Médecin chef
E-mail : Jerome.Biollaz@chuv.hospvd.ch

Centre de Transplantation d'Organes
Pr M. Pascual, Chef de service
E-mail : Manuel.Pascual@chuv.hospvd.ch

Lausanne, le 14 septembre 2005

ETUDE PHARMACOCINETIQUE ET PHARMACODYNAMIQUE DU VALGANCICLOVIR (VALCYTE®) CHEZ DES PATIENTS TRANSPLANTES D'ORGANE SOLIDE

Date d'envoi à la Commission d'Ethique : 31 mars 2005

Date d'envoi de la version révisée : 14 septembre 2005

Date prévue pour le début de l'étude : dès acceptation par la CE

Investigateur Responsable :
Dr Pascal Meylan , PD, Med-adjoint, Institut de Microbiologie et Division des Maladies Infectieuses, CHUV

Investigateurs en charge de l'étude :
Dr Jacques Fellay, Institut de Microbiologie et Division des Maladies Infectieuses, CHUV (aspects médicaux)
Nancy Perrottet, doctorante, Division de Pharmacologie et Toxicologie Clinique, CHUV (dosage du ganciclovir et analyses de données)

Autres investigateurs :
Pr Manuel Pascual, Centre de Transplantation d'Organes, CHUV
Dr Laurent Décosterd et Dr Thierry Buclin, Division de Pharmacologie et Toxicologie Clinique, CHUV
Dr John-David Aubert, Pr Darius Moradpour, Dr Charles Seydoux, Dr Jean-Pierre Venetz, Centre de Transplantation d'Organes
Pr Giuseppe Pantaleo, Division d'Immunologie et Allergie, CHUV

Lieu de l'étude : Centre Hospitalier Universitaire Vaudois (CHUV)

Promoteurs : cette étude est réalisée à l'initiative des investigateurs et ses coûts seront assumés par les trois unités correspondantes (IMU, PCL, CTO).

INTRODUCTION

L'infection par cytomégalovirus (CMV) est l'une des premières causes de morbidité et de mortalité chez les patients transplantés d'organe solide[1]. En plus de ses effets directs, ce virus cause une variété d'effets indirects qui augmentent en particulier le risque de rejet aigu ou chronique du greffon. [2]

Ces 10 dernières années, l'utilisation d'antiviraux pour le traitement ou la prophylaxie de l'infection à CMV a permis de réduire considérablement sa morbidité et mortalité. Jusqu'il y a peu, le valaciclovir et le ganciclovir étaient utilisés par voie orale en prophylaxie, alors que le ganciclovir était administré par voie intraveineuse pour traiter une infection déclarée à CMV. Le ganciclovir possède une activité contre le CMV plus élevée que le valaciclovir mais une biodisponibilité nettement inférieure (de l'ordre de 7%). Limité par ce facteur, un traitement utilisant de fortes doses orales de ganciclovir était nécessaire pour obtenir un taux plasmatique efficace dans le cadre d'une prophylaxie, mais qui demeurait insuffisant pour le traitement curatif d'une infection déclarée. Le valaciclovir, promédicament d'aciclovir, malgré une biodisponibilité bien plus élevée a une efficacité antivirale trop marginale contre CMV pour supplanter le ganciclovir intraveineux dans le traitement de cette infection[3]. Il n'existait donc aucun régime oral pour le traitement de l'infection à CMV déclarée chez les patients transplantés d'organe solide.

Récemment, un valyl-ester du ganciclovir, le valganciclovir a été développé comme prodrogue dotée d'une biodisponibilité nettement accrue (60%, soit environ 10 fois celle du ganciclovir). Après administration orale, la molécule est rapidement hydrolysée par des estérases de la muqueuse intestinale et du foie en ganciclovir. Ainsi 900 mg de valganciclovir génèrent une exposition systémique (aire sous la courbe) similaire à l'administration de 5mg/kg de ganciclovir intraveineux. Par conséquent, l'administration orale de valganciclovir offre une alternative des plus intéressantes pour le traitement de l'infection à CMV.

L'efficacité du valganciclovir a déjà été validée par des études contrôlées randomisées pour le traitement des rétinites à CMV chez des patients VIH positifs[4] ainsi qu'en prophylaxie d'une infection ou d'une maladie à CMV chez des patients transplantés du cœur, du foie ou du rein à haut risque de développer cette infection (donneur CMV séropositif/receveur CMV séronégatif)[5]. Par contre, l'utilisation du valganciclovir dans le traitement de l'infection à CMV déclarée chez des patients transplantés n'a pas encore été validée à ce jour. De plus, l'efficacité du valganciclovir n'a pas encore été prouvée en prophylaxie de l'infection à CMV après une transplantation pulmonaire. On ne sait pas non plus si les différences pharmacocinétiques entre le ganciclovir et le promédicament valganciclovir ont une influence sur l'efficacité clinique ou la tolérance, et si des situations comme une malabsorption, une mucoviscidose, une insuffisance rénale, ou des interactions médicamenteuses peuvent moduler significativement le profil du valganciclovir. Le ganciclovir est en outre un substrat important du transporteur rénal des anions organiques, un système impliqué dans l'élimination mais aussi potentiellement source de nombreuses interactions médicamenteuses[6]. Aucune publication sur la pharmacodynamie du ganciclovir ne rend compte d'une corrélation entre le taux plasmatique et les réponses virologique et clinique pour la maladie à CMV[7]. Par contre, le fabriquant a montré dans une étude clinique non publiée que l'aire sous la courbe du ganciclovir pouvait prédire la réponse clinique. In vitro, la corrélation a été clairement établie indiquant une concentration inhibitrice (IC_{50}) égale ou inférieure à 1 mg/l. D'autre part, une publication soulève l'interaction entre la réponse immunitaire par les cellules T et l'effet des antiviraux.[8]

Le but de la présente étude est de valider l'utilisation du valganciclovir pour le traitement et la prophylaxie de l'infection et de la maladie à cytomégalovirus chez les transplantés d'organe solide, tout en approfondissant les connaissances sur la pharmacocinétique, la relation concentration-effet et la sécurité de ce nouveau médicament. Tenant compte du fait que le valganciclovir est un promédicament du ganciclovir, nous proposons pour cette étude une approche alternative à un essai contrôlé randomisé qui nécessiterait des centaines de patients. Si au sein d'une même catégorie de patients, l'administration orale de valganciclovir donne des concentrations sanguines similaires à celles obtenues après une injection intraveineuse de ganciclovir et que ces taux permettent d'atteindre une réponse virologique similaire à celle suivant l'administration iv (environ –0.1 Log de diminution de la charge de DNA virale par jour, selon Emery et al[9] et nos propres données), nous pensons pouvoir légitimement déduire que le valganciclovir oral est équivalent au ganciclovir intraveineux dans le traitement de l'infection à CMV pour cette catégorie de patients. Parallèlement, si l'administration orale de valganciclovir induit des concentrations de ganciclovir supérieures à celles obtenues après une prophylaxie orale avec du ganciclovir et que ces taux préviennent efficacement une augmentation de la virémie, alors le valganciclovir pourra être considéré comme équivalent voire supérieur au ganciclovir oral pour la prophylaxie de l'infection à CMV dans cette catégorie de patients. La réalisation d'un essai randomisé rigoureux serait par ailleurs rendue difficile par le fait que l'utilisation du valganciclovir à la place du ganciclovir s'est déjà progressivement imposée dans ces indications, sur une base empirique, le traitement étant plus commode, ne requérant pas d'hospitalisation. Notre étude vise donc à valider une thérapie déjà introduite, en tenant compte des contraintes concrètes s'opposant à une étude plus rigoureuse. Afin d'optimiser l'utilisation du valganciclovir, la relation concentration-effet sera étudiée à l'aide d'un modèle pharmacocinétique-pharmacodynamique. De plus, des polymorphismes génétiques des transporteurs d'anions organiques ou d'autres systèmes pouvant potentiellement influencer la pharmacocinétique et la pharmacodynamie du valganciclovir seront étudiés. Finalement, pour investiguer les interactions entre le système immunitaire, les effets du médicament et l'infection, la réponse par les cellules T contre les antigènes de CMV sera suivie en parallèle avec la charge virale dès la fin de la prophylaxie.

L'étude a donc pour objectifs:

Principal :
- Valider la prescription du valganciclovir oral (à la place du ganciclovir intraveineux ou oral) pour la prophylaxie et le traitement de l'infection et de la maladie à cytomégalovirus pour toutes les catégories de transplantations d'organe solide.

Secondaires :
- Etudier la relation entre les concentrations sanguines de ganciclovir et l'efficacité anti-CMV à l'aide d'un modèle pharmacocinétique-pharmacodynamique (PK/PD), et identifier les facteurs pharmacocinétiques, virologiques et cliniques qui influencent cette relation.

- Déterminer les effets de la malabsorption, de la mucoviscidose, de l'insuffisance rénale et de l'inhibition du transport des anions organiques par divers médicaments sur la pharmacocinétique du valganciclovir et du ganciclovir, ainsi que sur la posologie à administrer.

- Evaluer la sécurité et la tolérance du valganciclovir oral au vu des concentrations obtenues

Cette étude permettra de confirmer qu'on peut étendre le bénéfice d'un médicament antiviral oral pour la prophylaxie et le traitement de l'infection à CMV chez des patients transplantés d'organe solide avec les avantages d'une administration per os versus intraveineuse. En particulier, les patients devraient bénéficier de la diminution des risques d'infection et du coût global du traitement, et d'un suivi ambulatoire moins contraignant qu'une hospitalisation. De plus, le traitement pourra être optimisé par l'identification des facteurs importants pour la pharmacocinétique et les réponses cliniques et virologiques. Finalement une individualisation du traitement sera possible dans certaines conditions particulières comme par exemple la mucoviscidose, l'insuffisance rénale, les interactions médicamenteuses et éventuellement les différences génétiques affectant les transporteurs des anions organiques.

PLAN DE L'ETUDE

Il s'agit d'une étude clinique ouverte non contrôlée (les données de la littérature sur le ganciclovir feront office de comparateur) pour la prophylaxie et le traitement de l'infection et de la maladie à CMV par le promédicament oral valganciclovir chez les patients transplantés d'organe solide.

Les patients recevront une prophylaxie de trois mois de valganciclovir (900 mg 1 fois par jour, sauf adaptation à la fonction rénale) quelque soit leur status sérologique (D+/R-, D+/R+, et D-/R+)[i] et seront suivis encore trois mois après l'arrêt de la médication. Si un patient devait présenter une virémie élevée lors du suivi virologique (>10'000-100'000 copies d'ADN de CMV par million de leucocytes), il sera alors traité avec une dose thérapeutique de valganciclovir (900 mg 2 fois par jour, sauf adaptation à la fonction rénale) jusqu'à ce qu'il montre une réponse clinique ou une charge virale inférieure à 10'000 copies/millions de leucocytes. Une prophylaxie secondaire sera alors commencée (900 mg 1 fois par jour, sauf adaptation à la fonction rénale) pour une période d'un mois. En moyenne, un patient devrait suivre l'étude sur une durée minimum de 6 mois.

L'étude est à ce jour monocentrique, mais il se pourrait que d'autres centres suisses avec des programmes de transplantation s'y associent ultérieurement.

SELECTION DES SUJETS

Tous les patients à risque de développer une infection à CMV (D+/R-, D+/R+, et D-/R+) seront éligibles pour l'étude. Dans la mesure du possible, l'étude leur sera présentée lors de la mise en liste (les personnes déjà sur liste seront contactées après l'acceptation du protocole). Dans de rares cas, l'étude sera proposée à l'introduction du valganciclovir (par exemple lors de greffe en urgence). Une partie des patients inclus dans l'étude se verront proposer la possibilité de participer à des investigations plus détaillées, à la condition de fournir un consentement éclairé supplémentaire. Une dizaine de patients volontaires seront nécessaires pour répondre aux objectifs de la sous-étude pharmacocinétique ; tout patient inclus dans l'étude principale pourra participer à cette sous-étude, à condition de donner son accord, et que le médecin-traitant n'y voie pas de contre-indication. Parmi les patients à risque élevé (D+/R-), environ dix prendront part à une sous-étude immunologique ; tout patient inclus dans l'étude principale pourra participer à cette sous-étude, à condition de donner son accord, et que le médecin-traitant n'y voie pas de contre-indication.

[i] Les différents status sérologiques sont les suivants :
D+/R- : donneur d'organe porteur du CMV et receveur naïf pour ce virus
D+/R+ : donneur et receveur d'organe porteurs du CMV
D-/R+ : donneur naïf et receveur porteur du CMV

Considération sur le recrutement :
Environ 50 procédures de transplantation d'organe solide sont effectuées au CHUV annuellement. Parmi elles environ 90% sont à risque de la maladie à CMV (D+/R-, D+/R+, et D-/R+), i.e. 45/an. Nous projetons donc de conduire cette étude sur deux ans, et d'inclure une centaine de patients environ.

Critères d'inclusion :
- patient adulte transplanté d'organe solide **(≥18 ans)**
- à risque de développer une maladie à CMV (D+/R-, D+/R+, et D-/R+)
- donnant son consentement éclairé

Critères de non-inclusion :
- impossibilité de donner un consentement éclairé
- intolérance connue au valganciclovir ou au ganciclovir

METHODE D'INVESTIGATION

Durant la prophylaxie, des échantillons sanguins seront prélevés chaque mois pour le dosage plasmatique du ganciclovir par chromatographie liquide à haute performance (taux résiduel juste avant la prise du médicament et taux au pic 3 heures après) ainsi que pour la mesure de la charge virale de CMV par PCR quantitative. Puis, durant les trois mois de suivi, des prélèvements seront effectués toutes les deux semaines pour suivre la virémie. Si un traitement devait être introduit chez un patient, la fréquence des prélèvements serait alors augmentée à une fois par semaine pendant cette période et ensuite diminuée à deux fois par mois durant la prophylaxie secondaire d'un mois, pour suivre les taux plasmatiques de ganciclovir et la charge virale. Le dosage virologique nécessite 2.5 ml de sang anticoagulé par EDTA et le dosage plasmatique de ganciclovir 5.5 ml sur Monovette EDTA. La quantité de sang prélevée au cours de l'étude de six mois sera de 56 ml par patient, ce volume sera plus élevé pour les patients devant être traité pour une infection déclarée. De plus, dans ce dernier cas, une prise d'urine sera effectuée avant le début du traitement pour une mise en culture afin d'analyser la souche virale.
Des tests génétiques seront effectués à partir des culots d'érythrocytes et de leucocytes pour les enzymes impliquées dans le métabolisme des médicaments (famille de gènes des cytochromes et apparentés), leur transport actif (famille « ATP-binding cassette » [ABC] et apparentés) et passif (famille «solute-carriers » [SLC] et apparentés). Les tests génétiques seront effectués après extraction de l'ADN de cellules sanguines lors d'un des prélèvements de routine. Le consentement aux tests génétiques ne sera pas une condition nécessaire à la participation au reste de l'étude.
Pour la sous-étude immunologique, 30 ml de sang seront prélevés pour déterminer la réponse de cellules T aux antigènes du CMV par une technique du laboratoire d'immunopathologie du SIDA utilisant les tétramères A2 et les tests de stimulation avec les peptides pp65. Cette analyse sera effectuée après la prophylaxie, toutes les deux semaines pendant deux mois ou si une infection se déclare, jusqu'à la fin de celle-ci.
Pour la sous-étude pharmacocinétique détaillée prévue sur un groupe d'une dizaine de patients, des échantillons sanguins de 5.5 ml seront prélevés juste avant puis 1, 2, 3, 4, 6, 8, 12 et 24 heures après l'administration de valganciclovir et les urines seront récoltées toutes les 4 heures sur 24 heures (avec un intervalle de 12 heures pendant la nuit). Donc 49.5 ml de sang seront prélevés pour ce collectif lors d'une journée. Le cas échéant, ces déterminations seront répétées à une deuxième occasion pour vérifier les effets de facteurs transitoires (par exemple une interaction médicamenteuse).

SURVEILLANCE MEDICALE

La présente étude ne nécessite pas une prise en charge médicale plus intensive que la prise en charge standard à l'exception de quelques prises de sang supplémentaires. En revanche dans les cas où un traitement est instauré, cette étude devrait permettre de valider l'emploi d'une médication orale avec un suivi ambulatoire, en remplacement d'une hospitalisation avec mise en place d'un accès veineux central.

ROLE DU PERSONNEL INFIRMIER

Le déplacement de la charge de travail des infirmières dans les services de lits vers celles des consultations ambulatoires a pour conséquence une nette diminution de la charge globale de travail.

MEDICAMENT

Le valganciclovir (Valcyte®, VGC) sera administré per os à une dose de 900 mg (2 comprimés de 450 mg 1 fois par jour) pour la prophylaxie de l'infection à CMV et de 1800 mg (2 comprimés de 450 mg 2 fois par jour) pour le traitement de l'infection. Ce promédicament, après ingestion, est rapidement hydrolysé en ganciclovir par les estérases intestinales et hépatiques. Le ganciclovir ainsi libéré circule en produisant une exposition systémique comparable à celle des doses standards intraveineuses, largement au-dessus de celle produite par le ganciclovir oral. Ceci explique l'efficacité du valganciclovir dans le traitement et non seulement dans la prophylaxie de l'infection à CMV. Le valganciclovir est commercialisé en Suisse avec les indications officielles « traitement d'induction d'une rétinite à CMV active chez les patients présentant un syndrome d'immunodéficience acquise (sida) et dont la vue semble être en danger ; traitement d'entretien au terme d'un traitement d'induction ainsi que pour le traitement d'une rétinite à CMV inactive chez les patients avec sida déclaré. »

Les études effectuées à ce jour montrent, comme il fallait s'y attendre, que le valganciclovir présente le même profil d'effets secondaires que le ganciclovir intraveineux, à l'exception des infections sur cathéter[10]. En particulier, il faut être attentif à la possibilité de toxicité hématologique, avec cytopénies. D'autres effets indésirables décrits incluent diarrhées, nausées, et céphalées.

EVALUATION DES RISQUES

Considérant que le valganciclovir n'est qu'un promédicament, valyl-ester du ganciclovir, il est très peu vraisemblable qu'il expose les patients à une toxicité autre que celle à laquelle ils seraient exposés avec un traitement habituel de ganciclovir oral ou intraveineux (voir par exemple l'innocuité comparable du valaciclovir et de l'aciclovir). En fait, la présente étude diminue le risque des patients en leur épargnant le risque substantiel lié à une voie veineuse centrale (hémorragie, pneumothorax, thrombose, infections).

Le risque de toxicité hématologique sera évalué par un suivi soigneux de la formule sanguine au cours du traitement, comme c'est le cas avec le ganciclovir intraveineux, ainsi que de la fonction rénale, afin de ne pas manquer de pratiquer les adaptations de dose nécessaires en cas de modification de cette fonction.

Dans la mesure où il s'agit d'une étude résultant de l'initiative d'un investigateur du CHUV, et non de l'industrie, c'est l'assurance responsabilité civile du CHUV qui couvrira les malades envers les risques liés à cette étude.

SOURCE DE FINANCEMENT

Le médicament, les contrôles de sécurité et virologiques seront pris en charge par l'hôpital (pour les patients hospitalisés) et par les assurances (pour les patients en ambulatoire).
Le coût des dosages de ganciclovir et de l'analyse des données (incluant le salaire d'un pharmacien-doctorant) seront couverts par les fonds de l'Institut de Microbiologie, de la Division de Pharmacologie Clinique et du Centre de Transplantation d'Organes.
Comparé aux frais du traitement standard, l'étude représentera une économie substantielle pour les assurances et l'hôpital en validant le remplacement d'un traitement intraveineux nécessitant une hospitalisation par un médicament administré per os avec un suivi ambulatoire possible. Par ailleurs, les coûts des médicaments sont égaux (ganciclovir intraveineux : 2568 Fr et valganciclovir 2677 Fr pour un traitement de 15 jours).
Aucune rétribution ne sera perçue par les investigateurs.

COLLABORATION AVEC LES PRATICIENS INSTALLES

Une collaboration n'est en principe pas requise, étant donné que tous les patients sont suivis par les médecins des consultations spécialisées s'occupant de patients transplantés. A noter que le dosage du ganciclovir, mis au point pour cette étude, pourra être mis à disposition des praticiens suivant d'autres patients (ex : rétinite à CMV) pour une aide à la décision.

DIVERS

L'information au personnel médical et paramédical sera assurée par l'investigateur responsable et le doctorant en charge du projet, relayé par les médecins des programmes de transplantation.

Dr Pascal Meylan
Institut de Microbiologie

Pr Manuel Pascual
Centre de Transplantation d'Organes

Dr Thierry Buclin
Division de Pharmacologie et Toxicologie Clinique

Nancy Perrottet
Division de Pharmacologie et Toxicologie Clinique

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Formulaire d'information

CENTRE HOSPITALIER UNIVERSITAIRE VAUDOIS

DEPARTEMENTS DE MEDECINE ET DE CHIRURGIE

Institut de Microbiologie
Pr A. Telenti, Médecin chef
E-mail : Amalio.Telenti@chuv.hospvd.ch

Division de Pharmacologie Clinique
Pr J. Biollaz, Médecin chef
E-mail : Jerome.Biollaz@chuv.hospvd.ch

Centre de Transplantation d'Organes
Pr M. Pascual, Chef de service
E-mail : Manuel.Pascual@chuv.hospvd.ch

Etude Pharmacocinétique et Pharmacodynamique du Valganciclovir (Valcyte®) chez des Patients Transplantés d'Organe Solide

ETUDE PRINCIPALE

Madame, Monsieur,

Suite à une transplantation d'organe, des médicaments immunosuppresseurs sont administrés pour diminuer le risque de rejet immunologique du greffon. Ce traitement entraîne une diminution des défenses contre l'infection. Il en résulte un risque non négligeable de réactivation de certains virus, comme le cytomégalovirus, normalement retenu silencieux grâce à l'activité du système immunitaire. Le développement de cette infection peut donner une maladie avec parfois des complications notamment pour le greffon. Afin de réduire au maximum ce risque, une prophylaxie avec un médicament antiviral est habituellement administré durant les trois premiers mois suivant la transplantation. Deux agents anti-infectieux, le ganciclovir et le valaciclovir, étaient utilisés par voie orale dans cette indication mais présentaient tous deux certaines limites. Récemment, un dérivé du premier, le valganciclovir (Valcyte®), a été développé pour augmenter l'absorption du médicament, permettant ainsi de diminuer la dose à administrer et de traiter une infection déclarée par voie orale plutôt que par voie intraveineuse. Il s'ensuit la possibilité d'éviter une hospitalisation au profit d'un suivi en ambulatoire. En raison des avantages apportés par ce nouveau médicament et de son efficacité prouvée par diverses études, de plus en plus de médecins l'utilisent dans ces indications. Déjà autorisé par la Communauté Européenne et par les Etats-Unis pour l'administration pour la prévention de cette infection chez certains patients transplantés, il n'a cependant pas encore été enregistré par les autorités sanitaires suisses pour le traitement d'une infection avérée, bien qu'il soit déjà utilisé dans cette situation.

Le but de la présente étude est donc de valider l'utilisation du valganciclovir pour le traitement et la prophylaxie de l'infection à cytomégalovirus chez les transplantés d'organe solide. Globalement, il s'agit de mesurer la concentration plasmatique du médicament et de vérifier que cette concentration atteint un niveau suffisant et non toxique. On contrôlera aussi son efficacité en suivant la quantité de virus dans le sang et la réponse clinique. De plus, une sous-étude est prévue chez une dizaine de patients pour suivre de plus près le profil des concentrations du médicament dans le sang (tout patient adulte consentant

pourra participer à cette sous-étude, avec l'accord de son médecin-traitant). Un autre collectif se verra par ailleurs proposer un suivi de la réponse immunitaire en parallèle avec les mesures de virémie dans le cadre d'une autre sous-étude (tout patient adulte consentant pourra participer à cette sous-étude, avec l'accord de son médecin-traitant).

D'une manière générale, la prise en charge post-transplantation ne sera que peu modifiée par l'étude. Quelques prises de sang supplémentaires seront nécessaires afin de doser le médicament (correspondant à un volume total de 33 ml). Au cas où une infection par cytomégalovirus se déclarait à distance du traitement préventif, le même médicament serait redonné, et des prises de sang supplémentaires seraient pratiquées pour suivre les concentrations (environ 44 ml). En principe, la fréquence des consultations pour le suivi ne sera pas augmentée par la participation à cette étude. Une analyse des gènes en rapport avec l'infection à cytomégalovirus et le devenir du médicament dans l'organisme sera effectuée à partir des globules du sang prélevé. Ces échantillons de sang seront conservés par le laboratoire de Pharmacologie et Toxicologie du CHUV et détruits après dix ans. Ils ne seront utilisés qu'aux fins scientifiques décrites ci-dessus, à l'exclusion de toute exploitation commerciale. Toute réutilisation poursuivant d'autres buts de recherche feront l'objet d'une nouvelle information particulière et d'un autre formulaire de consentement. En outre, vous pourrez en exiger la destruction en tout temps. La participation à l'étude n'est pas subordonnée obligatoirement à l'acceptation de ces tests génétiques.

Le médicament, selon les connaissances actuelles, présente le même profil d'effets secondaires que le ganciclovir, c'est à dire de possibles nausées, diarrhées et céphalées voir un abaissement du nombre de cellules sanguines. Comme il s'agit d'un médicament récemment développé, il n'est pas exclu qu'il puisse causer des effets indésirables encore inconnus cependant, compte tenu de sa grande ressemblance avec le ganciclovir, cela paraît très peu probable. Vous serez bien entendu surveillé de près durant toute la période de la prophylaxie et aussi dès l'arrêt de celle-ci pour une durée minimale de trois mois. Le suivi de vos concentrations sanguines de ganciclovir pourrait être bénéfique pour vous, dans la mesure où il permettra à votre médecin de vérifier que les doses que vous recevez sont efficaces et non toxiques. Malgré tout, si vous observiez un phénomène inhabituel dans votre état de santé, il ne faudrait pas manquer d'en informer votre médecin. La présente étude vous apporte un bénéfice important : si une infection à cytomégalovirus devait tout de même se déclarer, vous pourriez être traité en ambulatoire plutôt qu'être à nouveau hospitalisé, et sans qu'il soit nécessaire de vous poser un goutte à goutte. Cependant, vous pouvez à tout moment demander d'interrompre l'étude, sans que ceci ait une quelconque incidence sur la qualité des soins qui vous seront prodigués. En cas de préjudice subi dans le contexte de l'étude, vous seriez dédommagé intégralement. L'hôpital a conclu une assurance à cette fin. Le médecin-investigateur serait à votre disposition pour entreprendre les démarches nécessaires. Vous serez en outre informé en cas de nouvelles connaissances ayant trait à l'objet de l'étude.

En ce qui concerne les frais, le nouveau médicament (pratiquement au même coût que son prédécesseur), les analyses virologiques et de sécurité (examens de routine du suivi post-transplantation) seront facturés à l'hôpital pour les patients hospitalisés et à leur assurance pour les patients en ambulatoire. Les tests seront pratiqués de la même manière si vous ne participez pas à l'étude. Les examens supplémentaires à savoir les mesures de la concentration du médicament et les tests génétiques et immunologiques seront à la charge des investigateurs.

Les données récoltées dans cette étude sont confidentielles. Elles pourront cependant être consultées par des personnes impliquées dans votre prise en charge, à savoir les investigateurs et leurs collaborateurs, les autorités sanitaires, tous tenus à protéger votre confidentialité.

Si vous avez d'autres questions concernant cette étude, vous pouvez contacter le Dr Meylan (314 4098) ou votre médecin traitant s'occupant de votre transplantation.

Dr Pascal Meylan Nancy Perrottet

Formulaire de consentement

CENTRE HOSPITALIER UNIVERSITAIRE VAUDOIS

DEPARTEMENTS DE MEDECINE ET DE CHIRURGIE

Institut de Microbiologie
Pr A. Telenti, Médecin chef
E-mail : Amalio.Telenti@chuv.hospvd.ch

Division de Pharmacologie Clinique
Pr J. Biollaz, Médecin chef
E-mail : Jerome.Biollaz@chuv.hospvd.ch

Centre de Transplantation d'Organes
Pr M. Pascual, Chef de service
E-mail : Manuel.Pascual@chuv.hospvd.ch

Etude Pharmacocinétique et Pharmacodynamique du Valganciclovir (Valcyte®) chez des Patients Transplantés d'Organe Solide

ETUDE PRINCIPALE

Le soussigné:

- Certifie avoir été informé sur le déroulement et les objectifs de l'étude ci-dessus.
- Affirme avoir lu attentivement et compris les informations écrites fournies en annexe, informations à propos desquelles il a pu poser toutes les questions qu'il souhaitait.
- Accepte ou n'accepte pas que les gènes relatifs à la susceptibilité à l'infection à CMV, à la réponse immunitaire et au métabolisme et au transport du valganciclovir soient déterminés.
- Atteste qu'un temps de réflexion suffisant lui a été accordé.
- A été informé du fait qu'il pouvait interrompre à tout instant sa participation à cette étude sans préjudice d'aucune sorte.
- Consent à ce que les données recueillies pendant l'étude puissent être transmises à des personnes extérieures (autorités d'enregistrement), la confidentialité de ces informations étant sauvegardée.
- S'engage à informer le médecin responsable de tout phénomène inattendu pouvant survenir durant cette étude et à se conformer aux recommandations du médecin responsable de l'étude.

Le soussigné □ accepte donc de participer à l'étude mentionnée ci-dessus.
□ accepte les tests génétiques sur la susceptibilité à l'infection à CMV, à la réponse immunitaire et au métabolisme et au transport du valganciclovir

Lausanne, le ……

Nom et prénom du patient : ……

Signature du patient : ……

Nom et prénom du médecin : ……

Signature du médecin : ……

CHUV CENTRE HOSPITALIER UNIVERSITAIRE VAUDOIS
DEPARTEMENT DE MEDECINE

Centre de Transplantation d'Organes
Institut de Microbiologie
Division de Pharmacologie Clinique (PCL)
1011 Lausanne
Laboratoire PCL: BH 18.218, tél: 44 271,
Personne de contact : Nancy Perrottet (bip 744 861)

PATIENT
(ou étiquette DITO à code barre)

Nom, Prénom : ..... **Sexe :** ☐ F ☐ M
Date de naissance :
Service : ..... **Tél. :** .....
N° d'admission : .....

REQUÉRANT (médecin et service, téléphone)

Nom du médecin : .....
Service : ..... **Bip :** .....

DOSAGE PLASMATIQUE DE GANCICLOVIR ET ACICLOVIR

Médicament administré:

☐ Valganciclovir (Valcyte®) ☐ Ganciclovir (Cymevene®) ☐ Aciclovir (Zovirax®) ☐ Valaciclovir (Valtrex®)

INFORMATIONS INDISPENSABLES

PRÉLÈVEMENT : **Date effective :** 20 **Heure :** (h : min)

TRAITEMENT : **Date dernière prise :** 20 **Heure :** (h : min)

Date début traitement : 20
(ou dernier changement de posologie)

Dose : mg x/j (autre schéma, préciser sous commentaires)

Voie d'administration : ☐ oral ☐ i.v. **N° de lot :** .....

Indication au traitement : ☐ prophylaxie ☐ infection asymptomatique ☐ maladie : ..... ☐ autre : .....

Greffe : ☐ cardiaque ☐ rénale ☐ hépatique ☐ pulmonaire ☐ autre : .....

Autres diagnostics : ....

Fonctions anormales : ☐ cardiaque ☐ rénale ☐ hépatique ☐ aucune

Poids du patient : kg **Taille :** cm **Créatinine sérique :** µmol/L

AUTRES MÉDICAMENTS : ....
(pris sur la dernière semaine, ...
y.c. en automédication) ....
....
....

EFFETS INDÉSIRABLES OBSERVÉS SUR LA DERNIÈRE SEMAINE :

	absent	présent	grade (1- 4) (cf verso)		absent	présent	
1. Nausées, Vomissements :	☐	☐	.....	5. Anémie :	☐	☐	Hb : ..... g/l
2. Diarrhées :	☐	☐	.....	6. Leucopénie, Neutropénie :	☐	☐	Leuco : ..... G/l Neutro : ..... %
3. Toxicité cutanée :	☐	☐	.....	7. Thrombopénie :	☐	☐	Plaquettes : ..... G/l
4. Autres :	☐	☐	.....	8. Perturbation tests hépatiques :	☐	☐	ASAT : ..... U/l ALAT : ..... U/l
.....	☐	☐	.....				

COMMENTAIRES ET PRÉCISIONS (posologie particulière, demande spécifique, comorbidité, etc.) :

.....
.....

**Bon à joindre au prélèvement sanguin (*5.5 ml EDTA-K*),
et à envoyer sans délai au *Desk du LCC* – BH 18**

Version 1.0 / 24.12.2009 / PCL

DOSAGE PLASMATIQUE DE GANCICLOVIR ET ACICLOVIR

Le dosage de gancioclovir et aciclovir a été mis au point dans le cadre d'une étude clinique ayant pour objectif principal d'analyser la variabilité des concentrations plasmatiques de ganciclovir chez des patients transplantés d'organes. Il s'agit aussi d'étudier la relation entre les concentrations plasmatiques de ganciclovir et l'évolution clinique de l'infection à CMV. En d'autres termes, cette étude permettra de mettre en évidence si les récidives sont plus fréquentes chez les patients présentant de faibles taux de médicament et si les effets indésirables s'observent davantage chez les patients présentant des taux plus élevés.

Ainsi, la mesure des concentrations plasmatiques de ganciclovir pourrait améliorer l'emploi de ce médicament et la prise en charge des patients. Cependant, pour que les résultats soient utilisables, ils doivent nécessairement être interprétés au vu de toutes les données cliniques requises sur ce bon.
Merci de fournir en conséquence des informations complètes et précises.

D'autre part, le dosage du ganciclovir et de l'aciclovir est à disposition, à titre compassionnel, pour les patients ne participant pas à l'étude.

PRÉLÈVEMENT

- Monovette serum **5.5 ml EDTA-K** (bouchon bleu).
- **Délai de prélèvement optimal** des deux échantillons résiduel et à 3h :
  $T_{résiduel}$: avant dose suivante
  T_{3h} : environ 3 heures après la prise du médicament
- Un bon doit accompagner **chaque** prélèvement.
- Noter la date et l'**heure exacte** du **prélèvement sanguin** et de la **prise du médicament** sur chaque bon. Un décalage des prélèvements par rapport aux délais indiqués est acceptable pourvu que les heures **réelles** soient précisées.
- Envoyer immédiatement au desk du LCC, BH18, 1011 Lausanne-CHUV ; le prélèvement doit parvenir dans la journée.
- Pour les dosages demandés hors étude, un prélèvement résiduel seul peut être suffisant.

EFFETS INDÉSIRABLES : GRADES

Effet indésirable :	*Léger (grade 1)*	*Modéré (grade 2)*	*Sévère (grade 3)*	*Vital (grade 4)*
Nausées, vomissements	Pas d'interférence avec l'activité journalière, ou 1-2 épisodes/24h	Interférence partielle avec l'activité journalière, ou > 2 épisodes/24h	Empêche l'activité journalière, impose une hydration iv en ambulatoire	Consultation en urgence, hospitalisation, choc hypovolémique
Diarrhées	2-3 selles liquides, ou 400 g/24h	4-5 selles liquides ou 400-800 g/24h	≥ 6 selles aqueuses, ou 800 g/24h, ou impose une hydratation iv en ambulatoire	Consultation en urgence, hospitalisation, choc hypovolémique
Toxicité cutanée	Erythème, macules, papules, pigmentation, atrophie mineure, atteinte unguéale	Desquamation sèche, vésicules, prurit	Desquamation humide, exsudats, ulcérations	Dermatite exfoliative, nécrose imposant un traitement chirurgical

ESPACE RÉSERVÉ AU LABORATOIRE

REÇU LE : **Date :** | | | | | | | **Heure :** | | | (h : min)

TRAITEMENT : **Heure centrif. :** | | | | **Par :** | | **Visa :** | | **Volume :** | | | | µl

UNIVERSITE DE LAUSANNE

FACULTE DE BIOLOGIE ET DE MEDECINE
COMMISSION D'ETHIQUE
DE LA RECHERCHE CLINIQUE
RUE DU BUGNON 21-CH-1005 LAUSANNE

PROF. M. BURNIER
PRESIDENT

SECRETARIAT CENTRAL
TEL. +41 21 692 50 08
FAX +41 21 692 50 05

SOUS-COMMISSION I
PRESIDENT PROF. M. BURNIER
TEL. : +41 21 314 11 54

SOUS-COMMISSION II
PRESIDENT PROF. J.-P. GARDAZ
TEL. : +41 21 314 20 01

SOUS-COMMISSION III (PSYCHIATRIE)
PRESIDENT PROF. J. GASSER
TEL. : +41 21 643 64 65

Dr Pascal Meylan
Médecin-adjoint, PD et MER
Institut de Microbiologie et Division
des Maladies Infectieuses
CHUV
1011 Lausanne

Lausanne, le 20 septembre 2005

Protocole 94/05 : Etude Pharmacocinétique et Pharmacodynamique du Valganciclovir® (Valcyte) chez des Patients Transplantés d'Organe Solide

Monsieur et cher Collègue,

Je vous remercie de m'avoir adressé votre lettre du 14 septembre 2005 concernant l'étude susmentionnée.

Je vous remercie également de m'avoir transmis les documents suivants modifiés à la demande de Swissmedic:

- Protocole du 14 septembre 2005, version révisée
- Feuilles d'informations pour les patients (version révisée)
- Feuilles de consentement pour les patients (version révisée)

La Commission a pris note de ces nouveaux documents qui ne posent pas de problèmes éthiques. Vous pouvez donc les considérer comme **acceptés**.

En vous remerciant de m'avoir soumis ces documents, je vous prie de recevoir, Monsieur et cher Collègue, mes meilleures salutations.

Prof. Jean-Patrice Gardaz
Président de la Sous-Commission II

swissmedic

Réf.: dlw/dp
Tél. (dir.) +41 31 322 03 87
Fax +41 31 322 04 33
p. 1 de 1

Dr Thierry Buclin, PD, MER
Médecin adjoint
Centre Hospitalier Universitaire Vaudois (CHUV)
Pharmacologie et Toxicologie cliniques
Hôpital de Beaumont - 6ème étage
1011 Lausanne

Berne, le 28 septembre 2005

Notification de l'essai clinique "A pharmacokinetic and pharmacodynamic study of valganciclovir in solid organ transplant patients" (CE: 94/05)
N° de référence 2005DR2271

Docteur,

Nous vous remercions pour l'envoi des documents complémentaires requis par lettre du 12 septembre 2005 (reçus le 26 septembre 2005).

Veuillez trouver ci-joint un récépissé de notification dont le numéro d'ordre devra être mentionné lors de correspondance ultérieure.

L'essai clinique peut désormais commencer sans délai dans le canton de VD. Nous avons communiqué copie de la formule de notification et de l'agrément de la commission d'éthique aux autorités cantonales compétentes.

Veuillez agréer, Docteur, nos salutations distinguées.

Swissmedic, Institut suisse des produits thérapeutiques
Essais cliniques
Medical Reviewer GCP

V. Ditesheim

Docteur Véronique Ditesheim

Facture: Fr. 500.00 (suivra)
Ann: ment.

CENTRE HOSPITALIER UNIVERSITAIRE VAUDOIS

SOINS INTENSIFS ADULTES
DIVISION DE PHARMACOLOGIE CLINIQUE
CENTRE DE TRANSPLANTATION D'ORGANES

ADAPTATION POSOLOGIQUE DU VALGANCICLOVIR LORS D'HEMODIAFILTRATION VEINO-VEINEUSE CONTINUE CHEZ UN PATIENT DE SOINS INTENSIFS : ÉLABORATION D'UN MODELE PHARMACOCINETIQUE EN VUE D'UNE APPLICATION CLINIQUE

Investigateurs : N. Perrottet, Dr C. Willi-Robatel, Dr T. Buclin, Dr L. Décosterd, Pr J. Biollaz, Division de Pharmacologie et Toxicologie cliniques,
Dr M Berger , Soins Intensifs Adultes,
Dr O. Manuel, Pr M. Pascual, Centre de Transplantation d'Organes,
Dr P. Meylan, Maladies infectieuses, CHUV, Lausanne.

Sites d'études : Soins Intensifs Adultes, CHUV, Lausanne.

Date de soumission à la Commission d'Éthique : Etude générale approuvée en 1997 ; ce protocole spécifique est soumis le 8/6/2006.

Date du début de l'investigation : 8/6/2006

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1. Introduction et Buts du Projet

Les techniques d'épuration extra-rénale continue ont notablement amélioré le devenir des patients de soins intensifs durant ces dernières années. En effet, il n'est pas rare que les patients en état critique développent une insuffisance rénale aiguë, ou aggravent transitoirement une atteinte rénale préexistante, susceptible de compliquer leur prise en charge et leur pronostic. De plus, les séances d'hémodialyse classique sont mal tolérées par un organisme à la limite de ses possibilités d'adaptation, et leur réalisation se heurte souvent à des obstacles techniques. La mise au point de l'hémofiltration puis de l'hémodialyse continue a ainsi fourni aux intensivistes la possibilité d'une substitution à la fois douce, constante et largement réglable de la fonction rénale défaillante. Non seulement elle restaure partiellement un processus physiologique vital, mais elle élargit aussi la marge de manoeuvre pour ce qui est des apports liquidiens, nutritionnels et médicamenteux dont le patient a besoin.

L'insuffisance rénale en elle-même modifie clairement la pharmacocinétique de très nombreux médicaments, et cela non seulement pour les substances à élimination rénale

prépondérante, mais aussi pour des produits partiellement ou complètement métabolisés : l'efficacité des systèmes enzymatiques peut être modifiée, l'excrétion de métabolites éventuellement actifs ou toxiques, ou susceptibles d'être réactivés par la flore intestinale, est compromise, la liaison des produits aux composants du sang et des tissus est modifiée.. Pour certains médicaments faisant normalement l'objet d'une sécrétion ou d'une réabsorption tubulaires, la pharmacocinétique peut se modifier de manière non proportionnelle à la baisse de la filtration glomérulaire, la théorie du « néphron intact » étant prise en défaut. Ainsi, une adaptation posologique spécifique est indispensable pour beaucoup de médicaments administrés à l'insuffisant rénal, si l'on veut éviter l'apparition d'une toxicité liée à des taux circulants trop élevés. De nombreuses études ont été nécessaires pour proposer des directives d'individualisation des posologies dans ces circonstances. L'instauration d'une épuration extra-rénale artificielle complique encore la situation, et pose ainsi un réel problème aux intensivistes. Vu l'apparition récente de ces techniques, les données à disposition sont lacunaires, et certaines décisions thérapeutiques doivent être prises sur la base de raisonnements incertains. Pour les médicaments occasionnant des effets facilement mesurables, tels que les amines vasopressives, les anticoagulants, les analgésiques ou les sédatifs, la possibilité d'un rétrocontrôle limite la portée de ce problème. En revanche, pour les antiviraux, le clinicien n'a aucun moyen simple de savoir rapidement si les doses administrées sont adéquates, trop faibles, faisant craindre une efficacité insuffisante, ou trop élevées, pouvant occasionner une toxicité. Faut-il administrer les posologies standard de tel produit ? les adapter comme pour un insuffisant rénal ? en fonction de quelle échelle de gravité de l'atteinte rénale ? d'autres facteurs propres à l'hémodiafiltration doivent-ils encore être pris en compte ?

Ces considérations nous ont incités à mettre sur pied un projet de recherche clinique portant sur la pharmacocinétique des anti-infectieux lors d'hémodiafiltration continue chez les patients de soins intensifs. Il ne s'agit pas d'une étude unique, mais plutôt d'un faisceau d'études, visant un quintuple but :

- *décrire la pharmacocinétique* de certains anti-infectieux utilisés en soins intensifs dans les circonstances particulières où intervient une épuration extra-rénale continue ;
- tenter de *corréler les paramètres cinétiques* à certaines caractéristiques du patient (âge, mensurations, fonction rénale résiduelle, autres déficits des fonctions vitales, comédication, etc.), de la technique d'épuration utilisée (débits et pressions de filtration et de dialyse, filtre et appareillage utilisés, etc.) ;
- sur la base à la fois de ces observations et d'une revue extensive de la littérature, *élaborer un modèle pharmacocinétique* destiné à faciliter l'adaptation posologique, puis un *algorithme de calcul* visant à estimer le schéma de dosage requis dans une situation particulière pour obtenir des concentrations circulantes optimales, compte tenu des objectifs thérapeutiques ;
- *valider cliniquement le modèle* en comparant les concentrations circulantes prédites sur cette base et les taux effectivement mesurés, en observant également l'évolution du problème infectieux ;
- *développer des compétences* en matière de pharmacocinétique de l'épuration extra-rénale, afin d'affiner le raisonnement applicable à ce type de situation.

2. Considérations théoriques

Les processus qui interviennent dans la pharmacocinétique des médicaments chez les patients sous hémodiafiltration sont complexes. Pour l'analyse, il est commode de séparer les différentes voies de transfert empruntées par la substance administrée, en se référant au cadre général de la théorie pharmacocinétique, plus particulièrement au principe de la conservation de la masse : par unité de temps, le changement de la quantité de médicament présente dans l'organisme est la différence entre apports (perfusion) et élimination (par l'organisme et l'épuration artificielle). Dans l'élimination, il faut faire la part des processus physiologiques, à savoir l'excrétion rénale résiduelle et le métabolisme, et des processus artificiels, en distinguant la composante de filtration et la composante de dialyse qui coexistent à des degrés variables dans l'hémodiafiltration.

Absorption

Cette étude porte sur le valganciclovir, un promédicament oral du ganciclovir, qui est administré oralement (par le biais d'une sonde gastrique chez le patient de soins intensifs). Le valganciclovir étant administré par voie orale, les paramètres mesurés seront des clairances et volumes apparents (c'est-à-dire divisées par la valeur de biodisponibilité orale). Toutefois la clairance d'hémodiafiltration sera correctement estimée, compte tenu de la détermination des quantités éliminées par cette voie.

Distribution

En première approximation, pour des produits à distribution restreinte et rapide, tels que beaucoup d'anti-infectieux, la quantité présente dans l'organisme est simplement proportionnelle à la concentration circulante ; le coefficient de proportionnalité est le volume de distribution. Cependant, une analyse plus précise a toutes les chances de montrer que la distribution n'est pas instantanée, et que le *volume apparent* s'accroît progressivement d'une valeur *initiale* faible (≈ volume circulant et liquide extracellulaire des tissus bien perfusés) jusqu'à une valeur *terminale* plus élevée (≈ ensemble du liquide extracellulaire). Une cinétique bi- voire multicompartimentale doit alors être évoquée. De plus, la liaison éventuelle du médicament aux composants du sang doit être prise en compte si l'on veut modéliser la *concentration plasmatique libre*, qui est celle effectivement « vue » par les systèmes d'élimination physiologiques et artificiels.

Élimination rénale résiduelle

L'insuffisance rénale des patients étudiés n'est pas forcément complète. De fait, il persiste de cas en cas une certaine excrétion urinaire. En première approximation, cette élimination est d'ordre 1, et caractérisée par une *clairance* valant *la filtration glomérulaire résiduelle multipliée par la fraction plasmatique libre* du médicament :

$$CL_{\text{rénale résiduelle}} = f_u \cdot GFR_{\text{résiduelle}}$$

Cependant, cette approximation risque d'être passablement inexacte pour les médicaments subissant une sécrétion tubulaire active, tels que les pénicillines, pour lesquels on peut s'attendre à une clairance rénale résiduelle plus élevée (à moins qu'une atteinte tubulaire disproportionnée ne soit présente). Le transport actif étant saturable, l'élimination peut se révéler non linéaire à hautes concentrations. Des interactions médicamenteuses peuvent compliquer le problème (compétition pour le transport). Par ailleurs, certains médicaments

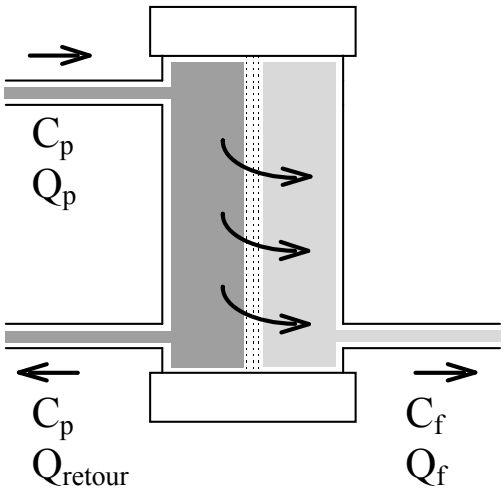
sont passivement réabsorbés à travers le système tubulaire, et leur clairance rénale devient alors fortement dépendante du débit urinaire (vitesse de l'urine dans les tubules) et du pH urinaire (modification de la polarité, et donc du passage transmembranaire du médicament). Pour des médicaments filtrés puis largement réabsorbés, on peut en arriver à la situation paradoxale où les procédés d'épuration extra-rénale éliminent plus efficacement la substance que le rein normal : les doses doivent alors être revues à la hausse par rapport aux posologies standard en cas d'hémodiafiltration. Ce serait le cas pour certains sulfamidés.

Elimination extrarénale

La plupart des médicaments sont partiellement éliminés par voie métabolique, si faible soit cette fraction métabolisée. Les études pharmacocinétiques chez le sujet sain permettent d'estimer une *clairance extrarénale*, qui est la différence entre la clairance systémique (obtenue à partir des concentrations plasmatiques seules) et la clairance rénale (obtenue à partir de l'excrétion urinaire). Cette clairance métabolique en conditions standard constitue cependant une estimation imprécise de la clairance extrarénale attendue chez le patient en hémodiafiltration : aux sources connues de variabilité (polymorphisme génétique, interactions médicamenteuses) s'ajoute l'influence spécifique de l'insuffisance rénale, déprimant volontiers les fonctions enzymatiques du foie, et celle d'autres atteintes physiologiques, telles que les perturbations hémodynamiques. Or l'importance relative de la clairance extrarénale, si elle est faible pour beaucoup d'anti-infectieux en conditions standard, devient plus nette en présence d'une atteinte rénale.

Hémofiltration

La composante de filtration dans l'hémodiafiltration représente la part de transport *convectif* emprunté par le médicament : à la faveur d'une différence de pression transmembranaire entre secteur sanguin et bain de dialyse dans le filtre, de l'eau plasmatique est filtrée, emportant les molécules trop petites pour être tamisées par le filtre :



Une représentation élémentaire indique que la vitesse d'élimination du médicament par filtration est égale au produit de sa concentration libre par le débit de filtration. On est donc en face d'un processus linéaire d'ordre 1, qu'on peut décrire par une clairance. Les spécialistes définissent le *coefficient de transférance* (tamisage ou « sieving ») d'un médicament donné dans un filtre donné comme le rapport de la concentration de médicament dans le filtrat sur la con-

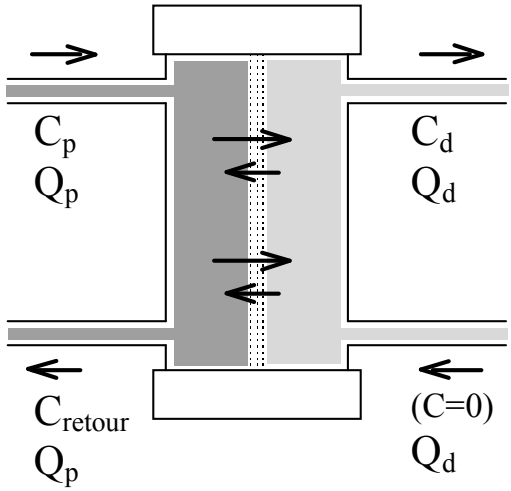
centration dans le plasma quittant le filtre. La *clairance de filtration* est alors égale à la transférance multipliée par le débit de filtration :

$$S_f = \frac{C_f}{C_p} \quad \Rightarrow \quad CL_f = S_f \cdot Q_f$$

Selon l'approximation évoquée ci-dessus, le coefficient de transférance d'un médicament pourrait être considéré comme égal à sa fraction libre dans le plasma. Différentes études confirment globalement la validité de cette approximation. Cependant, la mesure précise de ce coefficient en situation clinique révèle une influence spécifique du type de filtre utilisé (matériaux et géométrie) et du médicament (poids moléculaire et charges de surface). De plus, des interactions compliquées ont probablement lieu entre médicament, membrane et composants du sang : initialement, certains médicaments s'adsorberaient à la surface du filtre, étant ainsi soustraits du plasma sans se retrouver dans le filtrat, ce qui augmente le coefficient apparent ; progressivement, des composants du sang « encrassent » le filtre, entraînant soit une adsorption soit une répulsion du médicament, et modifiant ainsi sa transférance.

Hémodialyse

La composante de dialyse dans l'hémodiafiltration représente la part de transport par *diffusion* : à la faveur d'un gradient de concentration entre le secteur sanguin et le bain de dialyse, le plasma est progressivement « rincé » de son médicament :



A nouveau, le processus envisagé suggère une élimination d'ordre 1. La *clairance de dialyse* est décrite en relation avec le *coefficient de dialysance* du médicament considéré, dépendant également du filtre utilisé, défini comme le rapport entre concentration plasmatique à l'entrée du filtre et concentration dans le dialysat à la sortie du filtre. La clairance de dialyse est donc égale au produit du coefficient de dialysance par le débit de dialyse :

$$S_d = \frac{C_d}{C_p} \quad \Rightarrow \quad CL_d = S_d \cdot Q_d$$

Ici encore, en première approximation, on peut considérer le coefficient de dialysance égal à la fraction libre du médicament : en effet, le système à contre-courant, s'il fonctionne de manière optimale, permet que le dialysat quittant le filtre soit à l'équilibre avec le plasma

entrant dans le filtre. En pratique, les mesures du coefficient de dialysance des médicaments montrent souvent des valeurs plus faibles, en raison des interactions entre médicament, composants plasmatiques et membrane. Le poids moléculaire et les charges de surface des molécules de médicament influencent sa dialysance. Celle-ci tend également à diminuer quand on accélère le débit de dialyse, en diminuant du même coup le temps de contact entre sang et bain de dialyse. Le vieillissement du filtre a également un effet défavorable sur sa performance.

Interaction hémofiltration-hémodialyse

En pratique, les deux processus sont le plus souvent combinés à des degrés divers. L'hémofiltration permet l'élimination quantitative de volume circulant, avec les quantités correspondantes de solutés indésirables dans la circulation (déchets métaboliques, électrolytes). La mise en place d'une *prédilution* (perfusion de soluté de rinçage dans le sang avant son entrée dans le filtre) permet d'augmenter le transport convectif, plus efficace que la dialyse pour l'élimination des solutés, sans perte exagérée de volume. L'hémodialyse est utilisée pour éliminer plus spécifiquement les solutés, sans affecter le volume circulant ; le bain de dialyse peut d'ailleurs être utilisé pour apporter certains constituants à l'organisme. On définit un *coefficient de transférance* décrivant le rapport entre concentration dans le filtrat/dialysat à la sortie du filtre et concentration plasmatique à l'entrée du filtre. Hémofiltration et hémodialyse tendent à se combiner de manière hypo-additive, chacun des processus perturbant l'autre ; la clairance d'hémodiafiltration est donc inférieure à celle qu'on attendrait en additionnant la clairance de filtration et celle de dialyse :

$$S_a = \frac{C_{sortie(f+d)}}{C_p} \qquad \Rightarrow \qquad CL_a = S_a \cdot Q_{(f+d)} \quad \leq \quad S_f \cdot Q_f + S_d \cdot Q_d$$

De nouveau, le coefficient de transférance peut être approximé comme étant égal à la fraction libre du médicament dans le plasma, mais sa mesure révèle l'influence de plusieurs sources de variabilité.

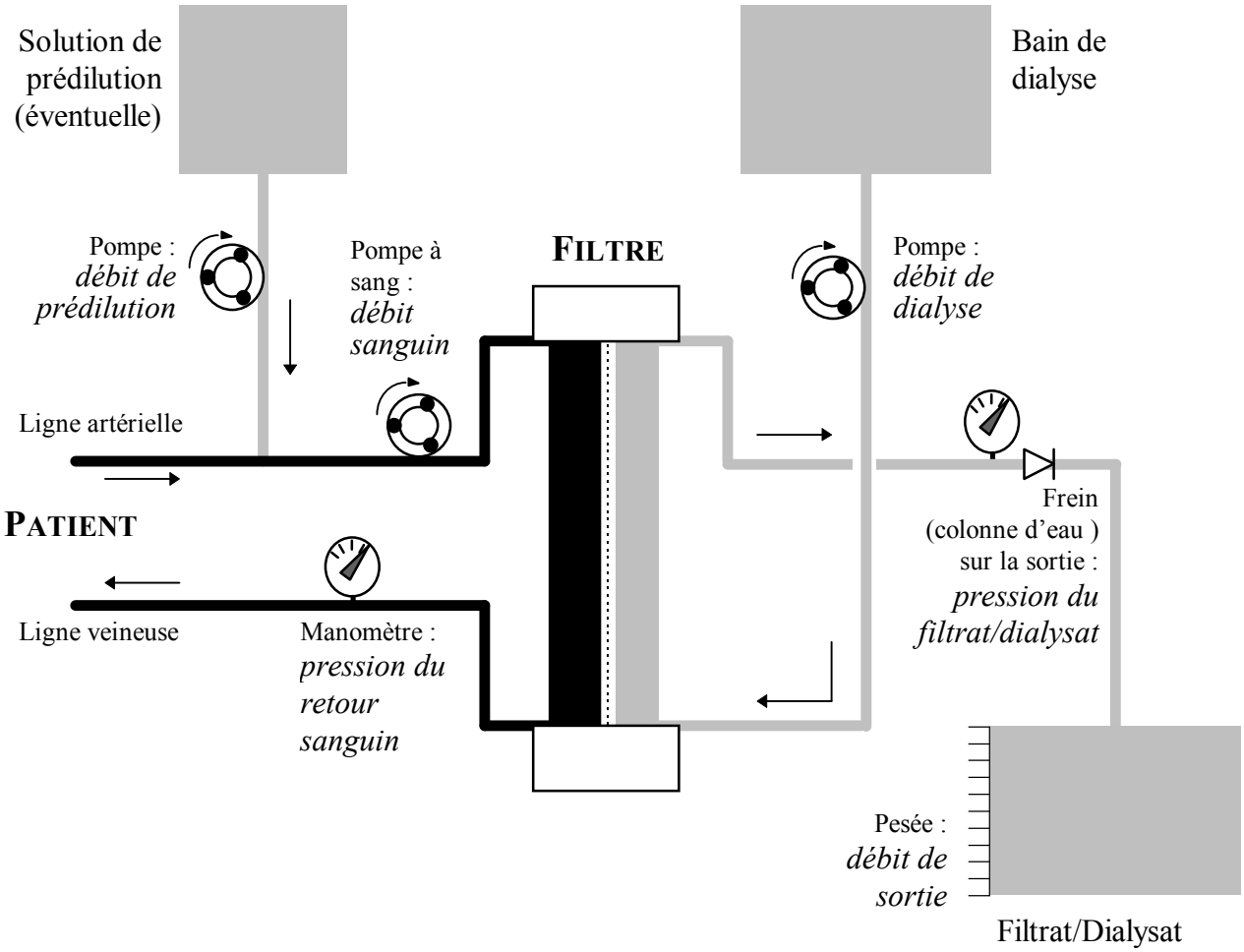
Considérations pharmacodynamiques

Le profil de concentrations circulantes d'un médicament en cas d'hémodiafiltration doit être considéré du point de vue de ses implications sur l'efficacité thérapeutique et la toxicité : l'adaptation posologiques à réaliser doit tendre à optimiser le rapport efficacité/risque. Les caractéristiques pharmacodynamiques des anti-infectieux doivent donc être invoquées pour déterminer les concentrations cibles. Pour des médicaments tels que les bêta-lactames et les glycopeptides, le plafonnement de l'effet bactéricide et l'absence d'effet post-antibiotique font qu'on tendra à maintenir durant le traitement des concentrations supérieures à 2-4 fois la CMI pour la bactérie visée, des pics plus élevés étant possiblement intéressants, en tout cas en début de traitement, pour favoriser la pénétration du médicament dans les sites infectés. Pour les quinolones et les aminoglycosides, l'effet post-antibiotique prolongé et des relations concentration-réponse plus progressives rendent intéressante l'obtention régulière de pics élevés ; la toxicité étant mieux corrélée aux concentrations résiduelles, celles-ci devront être maintenues basses en évitant l'accumulation. Ainsi, on choisira de cas en cas le type d'adaptation posologique, c'est-à-dire une modification de la dose unitaire, de l'intervalle entre doses, ou des deux. En situation clinique, la référence aux considérations pharmacodynamiques doit être mise en balance avec le type d'adaptation validée par l'expérience clinique, les deux approches ne conduisant pas toujours à des attitudes convergentes (cas de certains bêta-lactames pour lesquels la tradition consiste à adapter les intervalles plutôt que les doses).

3. Considérations Techniques

L'hémodiafiltration veino-veineuse continue sera seule étudiée dans ce projet. Cette technique est presque exclusivement utilisée à l'heure actuelle pour l'épuration artificielle chez les patients de soins intensifs présentant une atteinte de la fonction rénale.

Schématiquement, le dispositif utilisé est le suivant :



Les échanges sanguins se font via un double cathéter placé en général dans la veine fémorale. Une solution d'héparine est perfusée en continu dans le sang dès son entrée dans l'appareil, sous contrôle continu du temps de coagulation activé (Hemochron, valeur visée 180-200 s). Les filtres utilisés sont du type « fibres creuses » (Hospal Multiflow AN 69 HF, 1 m^2 ou 1.20 m^2) : le sang circule dans un faisceau de capillaires semi-perméables baignant dans le soluté de dialyse. Le volume mort du filtre est de 110 ml ; il doit être amorcé avec du soluté de dialyse avant la mise en route de l'appareil. Un filtre est en principe appelé à une durée d'utilisation de 24 à 48 h ; en pratique, on tend à prolonger l'utilisation des filtres autant que possible, sous contrôle de la clairance d'hémodiafiltration de l'urée.

L'appareillage standard (Prisma CFM) dispose de plusieurs pompes : la pompe à sang, réglant le *débit de passage du sang* à travers le filtre (généralement 100 à 150 ml/min), et la pompe à soluté de dialyse, réglant le *débit de dialyse* (en général 1 à 2 l/h). Un soluté de prédilution peut être adjoint au sang avant l'entrée dans le filtre, le *débit de prédilution* étant réglé à l'aide d'une pompe annexe. La pression du retour sanguin est mesurée en continu : elle dépend du débit sanguin, de la pression du bain de dialyse et de la résistance de la voie veineuse. La pression de sortie du filtrat/dialysat est réglée par un frein mis sur le circuit de sortie, sous la forme d'une surélévation du récipient de collection : la hauteur de la colonne d'eau fixe ainsi cette pression. La *pression transmembranaire* est considérée égale à la différence entre pression du retour sanguin et pression de sortie du filtrat/dialysat. Le *débit d'hémofiltration*

est mesuré comme la différence entre débit de dialyse et débit de sortie du filtrat/dialysat, ce dernier étant mesuré par pesée du récipient collecteur. L'appareil comprend un dispositif de contrôle asservi des pompes en fonction de valeurs de consignes choisies pour le débit de dialyse et le débit d'hémofiltration.

Les solutions de dialyse utilisées ont une composition standard (p. ex. Hemosol LG2), qui peut être modifiée au besoin par l'adjonction d'électrolytes (potassium, magnésium, bicarbonate...). Les lignes artérielle et veineuse de même que les tubulures afférente et efférente du dialysat sont pourvues de boutons à membrane perforable permettant les prélèvements à l'aiguille (conditions d'asepsie à respecter strictement). Le patient dispose généralement d'une voie veineuse complètement distincte de celle utilisée pour l'épuration, et par laquelle les médicaments sont administrés. Assez souvent, un cathéter artériel est en place et autorise des prises de sang. En l'absence d'anurie franche (débit urinaire < 300 ml/j), un cathéter vésical est en place et permet la récolte fractionnée des urines. Enfin, tous les patients de soins intensifs font l'objet d'un suivi rapproché des fonctions vitales (pression, pouls, température, paramètres ventilatoires), d'un suivi biologique fréquent (électrolytes, créatinine, urée, protéines plasmatiques), certains ayant un cathéter central en place (sonde de Swan-Ganz) permettant les mesures de débit cardiaque.

4. Organisation générale et Responsabilités

Concrètement, des études de dessin identique seront entreprises, portant successivement sur différents anti-infectieux. Le choix des anti-infectieux se restreindra aux substances utilisables en pratique chez les patients de soins intensifs.

La *réalisation des études auprès des patients* (sélection des patients, prélèvement d'échantillons et recueil de données) sera sous la responsabilité de l'**équipe des Soins intensifs de Médecine et de chirurgie**. Dans la mesure du possible, elle impliquera en première ligne un investigateur dédié afin que l'étude rajoute le moins possible de travail aux soignants. Celui-ci recevra toutefois une assistance de la part de l'équipe soignante des Soins intensifs pour la réalisation des gestes techniques (prélèvement d'échantillons) et le relevé des données médico-techniques.

Le *dosage de l'antiviral* dans les échantillons sera sous la responsabilité de la **division de Pharmacologie clinique**. Nous nous efforcerons de mettre à contribution un éventuel partenaire industriel capable d'effectuer ces dosages. La revue de littérature puis *l'élaboration du modèle* seront également effectués par la division de Pharmacologie clinique.

Toute l'investigation clinique sera effectuée en conformité avec les règles de bonnes pratiques cliniques.

5. Déroulement pratique de l'étude descriptive

Sélection des patients, consentement

Tout patient admis aux *Soins Intensifs*, chez qui l'indication à une *hémodiafiltration* aura été retenue sur la base des critères cliniques habituels, et qui devra recevoir un *anti-infectieux* entrant dans le cadre d'une des études de ce projet, sera en principe inclus.

Si l'état neurologique du patient le permet, un **consentement écrit** sera requis par un des investigateurs. Celui-ci se basera sur la formule d'information et de consentement fournie en annexe. L'information écrite sera complétée par des explications orales adaptées à la condi-

tion médicale et socio-culturelle du patient. L'original signé de la formule de consentement sera conservé dans le dossier médical du patient. Une copie sera confiée au patient, une autre sera versée au dossier d'investigation.Si l'état du patient ne permet pas la requête de son consentement, l'investigateur s'adressera à une personne de l'entourage proche, et leur demandera s'il est en mesure de présumer du consentement du patient à être inclus à l'étude. La même formule d'information et de consentement sera utilisée (voir annexe).

Au cas où aucun consentement ne pourrait être obtenu dans les délais requis pour pouvoir effectuer l'étude, les prélèvements seront effectués et l'information sera recueillie. Par la suite, dès que possible, le consentement sera formellement obtenu auprès du patient ou d'un proche, avant d'utiliser les prélèvements et les informations pour l'étude. Si cela devait se révéler définitivement impossible (p.ex. patient socialement isolé et décédé durant son hospitalisation), le consentement sera présumé pour autant que rien ne permette de suspecter que le patient se serait opposé à son inclusion s'il en avait eu la capacité.

D'autre part, le consentement du médecin en charge du patient à l'hôpital sera requis avant d'inclure le patient. Une copie du protocole sera mise au dossier des patients inclus, et l'obtention du consentement y sera consignée.

L'**investigation** proprement dite sera effectuée à partir du moment où le patient aura été mis sous *hémodiafiltration depuis au moins 24 h* ; le filtre devra être *âgé de 2 à 48 h*. Elle se déroulera sur un intervalle d'administration. Il est prévu que l'investigation puisse être effectuée **à deux reprises** chez chaque patient inclus, à au moins un jour d'intervalle, afin de pouvoir distinguer entre variabilité intra- et inter-patients, et d'augmenter la probabilité d'identifier les covariats ayant une influence significative.

Des patients atteints d'un *degré significatif d'anémie* ne seront *pas* inclus, afin de ne pas les exposer à une spoliation supplémentaire (limite : hémoglobine < 100 g/l chez les hommes, < 90 g/l chez les femmes). D'autre part, l'investigation ne sera *pas* entreprise si des *événements susceptibles de perturber l'étude* sont prévisibles (indication à une intervention chirurgicale, réanimation, instabilité gravissime des fonctions vitales...).

Traitements

Dans les études descriptives, l'inclusion du patient ne modifiera pas significativement le traitement qui aurait été donné hors étude. Tout au plus le choix d'un anti-infectieux pourra-t-il pencher pour un produit étudié, dans les situations où plusieurs alternatives thérapeutiques sont envisageables. Les doses d'anti-infectieux seront choisies sur la base des éventuelles recommandations du fabricant et de la littérature, et de la pratique habituelle des intensivistes.

L'hémodiafiltration sera poursuivie comme instituée. Simplement, le récipient de récolte du filtrat-dialysat sera changé juste avant l'administration de l'anti-infectieux, et on s'efforcera de garder constants les réglages de l'appareil (à moins évidemment que la situation clinique n'exige une correction).

Si le patient est porteur d'une sonde urinaire, celle-ci sera utilisée pour réaliser un prélèvement d'urine. Le sac de récolte sera changé juste avant l'administration de l'anti-infectieux. Il n'est pas prévu de sonder un patient du simple fait de son inclusion dans l'étude.

Les autres traitements en cours seront poursuivis sans changement.

Prélèvements

Les prélèvements suivants seront effectués :

- Juste avant l'administration de l'anti-infectieux, une fois le récipient de filtrat/dialysat et le sac d'urine changés : échantillons de sang de la voie artérielle et de la voie veineuse de l'appareil d'hémodiafiltration, et échantillon du filtrat/dialysat à la sortie du filtre.
- Après l'administration orale (dont on aura soigneusement relevé le temps) : 9 échantillons de la voie artérielle, de la voie veineuse et du filtrat/dialysat sur le reste de l'intervalle de dosage soit : 1 h, 2 h, 3 h, 4 h, 6 h, 12 h, 24 h, 36 h, 48 h après la prise de l'anti-infectieux.
- Les analyses de l'urée et de la créatinine sanguine nécessitées par le suivi du patient seront relevées sur la journée où l'investigation a lieu.
- Des échantillons de sang seront prélevés avant dose et à la fin de l'intervalle étudié en vue d'une détermination de la créatinine et de l'urée. De même, des échantillons de l'urine et du filtrat/dialysat seront conservés pour analyse de la créatinine et de l'urée. Ces prélèvements seront congelés en vue d'une analyse ultérieure.

Les échantillons de sang seront pris dans des Monovettes® à EDTA-K (noter que le patient est anticoagulé à l'héparine), de 2.7 ml, conservés à 4°C et centrifugées dans les 24 h suivantes (stabilité max 48 h). Le plasma sera récolté dans des tubes de polypropylène et congelé à -20°C. L'urine et le filtrat-dialysat seront mis dans des tubes similaires et congelés de même.

Une investigation impliquera donc le prélèvement de **20 échantillons de sang** veineux (10 sur la ligne artérielle, 10 sur la ligne veineuse), équivalent à environ 50 ml de sang au total. A cela s'ajoutent **11 échantillons de filtrat/dialysat**, **1 aliquote** de la solution injectée, et éventuellement **1 échantillon d'urine**.

Il ne sera pas nécessaire d'effectuer les échantillons aux temps exacts mentionnés ci-dessus. En revanche, le relevé exact de l'heure et de la minute *réelles* de chaque prélèvement sera de première importance.

Informations relevées

Un cahier d'observation sera élaboré, afin de relever pour chaque investigation les données suivantes :

- Consentement obtenu auprès du patient ou d'un proche.
- Caractéristiques démographiques du patient : âge, sexe, race, poids, taille (à défaut par estimation oculaire).
- Diagnostics cliniques, score de gravité (SAPS II), insuffisance cardiaque, respiratoire, hépatique, présence d'oedèmes et/ou d'épanchements, présence et débits d'éventuels drains (moyenne durant l'intervalle étudié).
- Médicaments administrés durant les 24 h précédentes et sur l'intervalle étudié, en particulier composition et débit des perfusions et l'héparine perfusée par l'appareil d'hémodiafiltration.
- Pression, température, fréquence respiratoire, rythme cardiaque, pression veineuse centrale, résultats de gazométrie artérielle et saturation d'oxygène par pulsoxymétrie durant l'intervalle étudié, à chaque temps de prélèvement.

- Si patient intubé : paramètres ventilatoires, fraction d'oxygène, échanges gazeux, pressions appliquées (moyennes durant l'intervalle étudié, en relevant d'éventuels changements aigus).
- Si sonde de Swan-Ganz en place : débit cardiaque, pressions veineuse, artérielle pulmonaire et capillaire bloquée durant l'intervalle étudié (moyennes durant l'intervalle étudié, en relevant d'éventuels changements aigus).
- Si sonde urinaire : débit durant l'intervalle étudié (mesure et échantillon).
- Données de laboratoire : créatinine, urée, hématocrite, protéines, albumine, bilirubine.
- Résultats microbiologiques, en particulier antibiogrammes des germes mis en évidence (en obtenant du laboratoire les CMI correspondantes).
- Détail des doses précédentes d'anti-infectieux, avec heures d'administration (début et fin de perfusion).
- Détail de l'administration de la dose en début d'intervalle étudié (heure et minute de début et de fin de perfusion, volume perfusé, concentration nominale de l'infusat en anti-infectieux).
- Historique de l'hémodiafiltration avant l'investigation (début, débits moyens, changements de filtre).
- Détail des réglages de l'appareil d'hémodiafiltration, relevés *avant l'injection d'antibiotique*, *à 60 min puis après chaque prélèvement d'échantillons* : débit sanguin, débit et composition d'une éventuelle solution de prédilution, pression du sang à la sortie du filtre (et à l'entrée si disponible), débit et composition de la solution de dialyse, pression du bain de dialyse à la sortie du filtre (calcul de la pression transmembranaire), débit du filtrat/dialysat, type d'appareil et de filtre utilisés, âge du filtre.
- Horaire précis des prélèvements, relevé des volumes mesurés.
- Évolution clinique du patient, en particulier des infections traitées, et surveillance d'éventuels signes de toxicité liée à l'anti-infectieux.

Mesure des taux d'anti-infectieux

Une méthode analytique appropriée a été développée et validée selon les règles d'assurance de qualité en usage dans le laboratoire de Pharmacologie clinique. Des contrôles de qualité internes sont effectués.

Les échantillons récoltés seront dosés à la fin de l'étude descriptive.

Analyse des données

Les résultats de l'étude descriptive seront d'abord analysés selon des méthodes simples : détermination de la demi-vie, de l'aire sous la courbe durant l'intervalle de dosage et de la clairance systémique s'y rapportant, du coefficient de transférance aux différents temps de prélèvement, et de la fraction excrétée par hémodiafiltration et par excrétion urinaire.

Une modélisation pharmacocinétique sera ensuite effectuée. Un modèle de type « physiologique » sera appliqué à l'appareil d'hémodiafiltration, afin de corréler sa performance dans l'élimination de l'anti-infectieux aux paramètres de réglage de l'appareil. L'influence de

certains covariats pertinents sera recherchée, tant parmi les données touchant à l'hémodiafiltration elle-même que parmi les informations cliniques relevées.

Une fois un modèle satisfaisant élaboré, il sera confronté avec les données de la littérature et les concentrations-cibles proposées pour l'anti-infectieux. Un module d'adaptation a priori des posologies sera proposé, intégrant les différents covariats dont l'influence aura été retenue.

6. Évaluation des Risques

Les études prévues dans ce projet, relativement intensives, consisteront purement en des prélèvements d'échantillons et des relevés d'information. Le traitement des patients ne sera pas modifié, hormis l'éventualité d'un choix d'anti-infectieux différent de celui qui aurait été fait en dehors du projet. Comme déjà souligné, le choix d'introduire un anti-infectieux compris dans les substances étudiées plutôt qu'un autre ne sera fait que dans les situations où les deux alternatives seront jugées équivalentes au plan thérapeutique. Tous les anti-infectieux peuvent avoir des effets indésirables, et cette probabilité étant grossièrement équivalente à l'intérieur d'une classe donnée, il n'y a pas lieu d'attendre une majoration du risque du fait de ce choix. Chaque investigation représentera le prélèvement d'environ 50 ml de sang au patient. Ce volume est négligeable en l'absence d'anémie significative, qui représente un critère de non-inclusion. Les manipulations supplémentaires des lignes sanguines effectuées pour les prélèvements entraînent un risque d'accidents d'asepsie ; des procédures de désinfection rigoureuse des orifices de prélèvements sont à même de minimiser ce risque. Les procédures d'hémodiafiltration ne seront pas affectées autrement par l'étude ; en particulier, il n'y aura pas de standardisation particulière, les différences de réglage de l'épuration artificielle permettant d'apprécier leur impact en situation clinique.

Thierry Buclin
Médecin associé
Division de Pharmacologie clinique

Jérôme Biollaz
Médecin-chef
Division de Pharmacologie clinique

CENTRE HOSPITALIER UNIVERSITAIRE VAUDOIS

SOINS INTENSIFS ADULTES,
DIVISION DE PHARMACOLOGIE CLINIQUE

Dr M.-D. Schaller, Médecin-Chef et Responsable de l'Etude
Dr R. Chioléro, Médecin-Chef
Pr J. Biollaz, Médecin-Chef

Etude clinique : Adaptation Posologique de différents Anti-infectieux lors d'Hémodiafiltration continue chez des patients de Soins Intensifs.

FEUILLE D'INFORMATION ET DE CONSENTEMENT

☞ *A remplir par l'investigateur en présence du patient ou d'un proche :*

Concerne : Mme M. ..... (Nom, Prénom du Patient)

Information et requête du consentement par : ..... (Investigateur)

Date : ..... Lieu : .....

Information :

La personne précitée est actuellement hospitalisée aux Soins Intensifs pour une affection grave. Du fait d'une atteinte rénale, une *hémodiafiltration continue* a du être mise en place, à l'aide d'un appareil destiné à filtrer le sang (rein artificiel). D'autre part, l'état de cette personne nécessite que des *Anti-infectieux* lui soient administrés. Ces traitements ont été décidés par l'équipe médicale en charge du patient, selon les standards actuels de la pratique médicale.

Or les connaissances disponibles sur l'emploi des nouveaux anti-infectieux lors d'hémodiafiltration continue sont encore incomplètes. Ce problème préoccupe des spécialistes de l'hôpital, qui ont mis sur pied une étude visant à mieux préciser les doses d'anti-infectieux à donner à ces malades.

La personne précitée pourrait être incluse dans cette étude. Cela équivaudrait à réaliser une série de prélèvements sanguins à partir de l'appareil d'hémodiafiltration (au total 40), des prélèvements de liquide de dialyse et d'urine, et à relever diverses informations à partir des dossiers médical et infirmier. Une personne serait spécifiquement chargée de l'investigation, en collaboration avec l'équipe soignante des Soins Intensifs. Il n'y aurait pas d'autre interférence entre l'étude et la prise en charge médicale du patient. Le protocole de cette étude a été approuvé par le Comité d'Ethique de la Faculté de Médecine de Lausanne.

Le but de cette information est de permettre au patient, ou à une personne qui lui est proche et qui peut se prononcer en son nom, de consentir à ce qu'il soit inclus dans l'étude. Ce consentement pourra être retiré librement en tout temps. L'acceptation ou le refus n'auront aucune incidence sur la prise en charge médicale fournie au patient. Si aucun consentement n'a pu être donné à temps, les investigateurs auront effectué les prélèvements le cas échéant, mais s'engagent à ne les utiliser dans le cadre de l'étude qu'une fois le consentement obtenu.

Consentement :

La personne précitée a reçu l'information ci-dessus, ainsi que des explications orales de la part de l'investigateur, à propos de l'étude. Elle consent à son inclusion, c'est-à-dire à ce que des prélèvements soient effectués, et à ce que des informations le concernant soient relevées, pour les besoins de l'étude, aux conditions spécifiées ci-dessus.

☞ *A signer par le patient :*

Signature : .....

Au cas où le patient précité n'est pas capable lui-même de recevoir l'information et de donner son consentement, la personne soussignée affirme faire partie de ses proches et être en mesure de présumer du consentement du patient à être inclus dans l'étude.

☞ *A signer par le proche :*

Nom, Prénom : ..... Signature : .....

☞ *Original à conserver dans le dossier médical du patient. Une copie va au patient ou au proche, une à l'investigateur.*

UNIL | Université de Lausanne
Faculté de biologie
et de médecine

COMMISSION D'ÉTHIQUE
DE LA RECHERCHE CLINIQUE
RUE DU BUGNON 21-CH-1005 LAUSANNE

PROF. M. BURNIER
PRESIDENT

SECRETARIAT CENTRAL
TEL. +41 21 692 50 08
FAX +41 21 692 50 05

SOUS-COMMISSION I
PRESIDENT PROF. M. BURNIER
TEL. : +41 21 314 11 54

SOUS-COMMISSION II
PRESIDENT PROF. J.-P. GARDAZ
TEL. : +41 21 314 20 01

SOUS-COMMISSION III (PSYCHIATRIE)
PRESIDENT PROF. F. STIEFEL
TEL. : +41 21 314 02 34

Dr. Thierry Buclin
Médecin associé
Division de Pharmacologie et
Toxicologie cliniques
CHUV
1011 Lausanne

Lausanne, le 26 juin 2006
MB/cc

Concerne : Extension de l'étude CVVHD au Valganciclovir

Cher Collègue,

J'ai bien reçu vos documents datés du 8 juin 2006, documents qui ont été discutés de manière informelle avec les membres de la Commission en date du 19 juin 2006. La Commission est d'avis qu'il serait plus judicieux, effectivement, de rédiger un protocole générique sur l'adaptation posologique des antibiotiques et des antiviraux lors d'hémodiafiltrations veino-veineuses continues chez les patients de soins intensifs. De cette manière vous ne seriez pas obligé de faire une demande spécifique pour chaque nouveau patient concerné par de telles mesures.

Je vous propose donc de nous soumettre un protocole un peu plus large qui irait dans ce sens. En ce qui concerne la patiente traitée au Valgangiclovir, la Commission ne s'oppose pas à ce que l'étude soit réalisée.

Pour ce qui me concerne, dans la mesure où il s'agit d'une technique d'hémodialyse, je pense que la collaboration du néphrologue pourrait être également intéressante dans ce projet.

En restant à ta disposition pour discuter de ce projet, je te prie de recevoir, cher Ami et cher Collègue, mes meilleures salutations.

Prof. M. Burnier
Président

Adaptation posologique de différents antiviraux lors d'hémodiafiltration veino-veineuse continue chez des patients de soins intensifs

Antiviral testé: ☐

Nom: ☐
Prénom: ☐ **No. de patient dans l'étude:** ☐
Etape: 1 ☐ 2 ☐ *(min 24h entre les 2 étapes)*

Date de naissance: ☐ Sexe: ☐

Service: ☐ Date actuelle: ☐
Médecin resp.: ☐ Date admission CHUV: ☐
Date admission S.I.: ☐

Consentement du patient/proche:
Information donnée au patient le ☐ à un proche ☐ le ☐
Consentement écrit obtenu du patient le ☐ d'un proche ☐ le ☐
Copie à annexer à ce document, original dans le dossier médical

Evaluation clinique

Mensurations: Poids: ☐ kg balance ☐ anamnèse ☐ estimation ☐
Taille: ☐ cm toise ☐ anamnèse ☐ estimation ☐

Race: caucasien ☐ africain ☐ asiatique ☐ métis ☐

Description de l'histoire clinique: _____

Commentaires

Score d'état clinique SAPS II: ☐
(estimation par l'équipe soignante)

Score d'atteinte cardiaque: ☐
(0= aucune 1= infraclinique 2= symptomatique 3= handicapante 4= menace vitale)
(selon médecin en charge du patient)

Score d'atteinte respiratoire: ☐
(0= aucune 1= infraclinique 2= symptomatique 3= handicapante 4= menace vitale)
(selon médecin en charge du patient)

Score d'atteinte hépatique: ☐
(0= aucune 1= infraclinique 2= symptomatique 3= handicapante 4= menace vitale)
(selon médecin en charge du patient)

Présence d'oedèmes et/ou épanchements: ☐
(0= aucun 1= discrets 2= moyens 3 = marqués 4= anasarque)
(selon médecin en charge du patient)

Présence de drains: oui ☐ non ☐ nombre: ☐

Débit moyen des drains durant la période d'observation: ☐ ml/h au total
(Si présence de drains, noter le niveau de chaque collection en début de période, puis en fin de période, au verso de la feuille)

Date: ☐ **Etape:** ☐ **No. de patient dans l'étude:** ☐

Patient intubé: oui ☐ non ☐
valeurs moyennes durant la période obser *(relever les valeurs en début de période, puis revoir feuille d'infirmière en fin de période)*

commentaires /changements aigus

Débit ventilatoire: ☐ l/min

Fraction d'oxygène: ☐ %

PEEP: ☐ cm H2O

Sonde de Swan-Ganz: oui ☐ non ☐
valeurs moyennes durant la période obser *(relever dernière mesure, puis évent. mesure durant période pour obtenir une moyenne représentative)*

commentaires /changements aigus

Débit cardiaque: ☐ l/min

Index cardiaque: ☐ l/min/m2

Pression veineuse centrale: ☐ mmHg
(Oreillette droite)

Pression artérielle pulmonaire moyenne: ☐ mmHg

Pression capillaire pulmonaire bloquée: ☐ mmHg

Résultats de laboratoire
Dernières mesures avant la période d'observation

Créatinine: ☐ µmol/l

Urée: ☐ mmol/l

Hématocrite: ☐ %

Hémoglobine: ☐ mg/l *non inclusion dans l'étude si Hb < 100mg/L chez un homme, < 90mg/L chez une femme*

Protéines: ☐ g/l

Albumine: ☐ g/l

Bilirubine directe: ☐ µmol/l

Bilirubine totale: ☐ µmol/l

Gazométrie artérielle
Dernières mesures avant la période d'observation
(relever dernière mesure, puis évent. mesure durant période pour obtenir une moyenne représentative)

Commentaires

pH: ☐
pO2: ☐ mmHg
pCO2: ☐ mmHg

Date: ☐ **Etape:** ☐ **No. de patient dans l'étude:** ☐

Résultats microbiologiques *(S= sensible I= indifférent R= résistant et/ou ø mm)*

		Ab. testés															
Germe	localisation																ømm pour Ab étudié

Commentaires

Médicaments administrés dans les dernières 24 heures (y compris perfusions)
Pour être inclu dans l'étude, le patient devrait avoir reçu au moins 2 doses identiques de l'antiviral testé.

Sédatifs et analgésiques:

Spécialité	DCI	Voie administration	Doses unitaires		Perfusion	Date introduction
			Dose (moyenne)	Fréquence (sur 24 h)	Débit de dose (moyenne 24 h)	

Amines, cardioactifs vasoactifs:

Spécialité	DCI	Voie administration	Doses unitaires		Perfusion	Date introduction
			Dose (moyenne)	Fréquence (sur 24 h)	Débit de dose (moyenne 24 h)	

Date: ☐ **Etape:** ☐ **No. de patient dans l'étude:** ☐

Antiinfectieux:

Spécialité	DCI	Voie administration	Doses unitaires Dose (moyenne)	Doses unitaires Fréquence (sur 24 h)	Perfusion Débit de dose (moyenne 24 h)	Date introduction

Antiviral étudié, voir précisions ci-dessous

Anticoagulants:

Spécialité	DCI	Voie administration	Doses unitaires Dose (moyenne)	Doses unitaires Fréquence (sur 24 h)	Perfusion Débit de dose (moyenne 24 h)	Date introduction

Antiulcéreux et autres

Spécialité	DCI	Voie administration	Doses unitaires Dose (moyenne)	Doses unitaires Fréquence (sur 24 h)	Perfusion Débit de dose (moyenne 24 h)	Date introduction

Solutés:

Spécialité	DCI	Voie administration	Doses unitaires Dose (moyenne)	Doses unitaires Fréquence (sur 24 h)	Perfusion Débit de dose (moyenne 24 h)	Date introduction

Commentaires: _____

Date: ☐ **Etape:** ☐ **No. de patient dans l'étude:** ☐

Apports entériques

Spécialité	Composition	Voie administration	Doses unitaires Dose (moyenne)	Doses unitaires Fréquence (sur 24 h)	Perfusion Débit de dose (moyenne 24 h)	Date introduction

Antiviral étudié

heures les plus exactes possible des dernières doses (48 h. écoulées)

date:						
heure administration:						
dose:						
(duréee perf:)						

Sonde urinaire oui ☐ non ☐

Débit urinaire moyen durant les ☐ dernières h: ☐ ml/h

Commentaire: _____

Hémodiafiltration:

Début de l'hémodiafiltration: le ☐ à ☐ h:min

Pour cette étude le patient doit être sous hémodiafiltration depuis au moins 24 h.

Type d'appareil utilisé: ☐ No. d'inventaire: ☐

Type de filtre utilisé: ☐ surface: ☐ m2

Pour cette étude le filtre doit être âgé de 2 à 48 h.

Dernier changement de filtre le ☐ à ☐ h:min

Nombre de filtres utilisés avant celui-ci: ☐

Solution de prédilution: oui ☐ non ☐

Composition de la solution de prédilution: _____

Composition du bain de dialyse: _____

Conditions avant période d'observation (moyenne sur ☐ dernières heures):

		Commentaires
Débit sanguin moyen:	☐ ml/min	_____
Débit moyen sol. prédilution:	☐ l/h	
Débit moyen de dialyse:	☐ l/h	
Pression moyenne du retour sanguin:	☐ mm Hg	
Pression moyenne du filtrat/dialysat:	☐ mm Hg	

(évent. hauteur urimètre-filtre cm X 0.75)

Procédure

Date: ☐ **Etape:** ☐ **No. de patient dans l'étude:** ☐

H. théorique	H. réelle	A faire	Remarques (no de renvoi)
Pré dose	☐ :	**Changement:** récipient filtat/dialysat	
	☐ :	sac urine	
	☐ :	**Prélèvements:** sang voie artérielle + 1 tube urée/créatinine	*(2.7 + 1.5 ml)*
	☐ :	sang voie veineuse	*(2.7 ml)*
	☐ :	filtrat/dialysat (sortie du filtre)	*(2.7 ml)*
		Appareil: Débit sanguin: ☐ ml/min	
		Débit sol. prédilution: ☐ l/h Débit postdilution: ☐ l/h	
		Débit de dialyse: ☐ l/h Prél. patient: ☐ l/h	
		P. du retour sanguin: ☐ mm Hg Débit UF: ☐ l/h	
		P. du filtrat/dialysat: ☐ mm Hg *(évent.hauteur urimètre-filtre x0.75)*	
		Patient: Pression sys/dias: ☐ / ☐ mmHg T°: ☐ °C	
		Fréquence resp: ☐ rpm P.veineuse centrale: ☐ mm Hg	
		Rythme card: ☐ bpm Saturation O2: ☐ % *(pulsoxymétrie)*	
	☐ :	**Bilan:** niveaux apports (sans pré/postdilution): ☐ l	
		niveau sol. prédilution: ☐ l *(détail au verso)*	
		niveau sol. postdilution: ☐ l	
t = 0	☐ :	**Administration antiviral testé.**	
		Dose: ☐ mg	
		Mode d'administration: ☐	
t = 1H	☐ :	**Prélèvements:** sang voie artérielle	*(2.7 ml)*
☐ :	☐ :	sang voie veineuse	*(2.7 ml)*
	☐ :	filtrat/dialysat (sortie du filtre)	*(2.7 ml)*
	☐ :	aliquot de l'injectat (dans tubulure)	
t = 2 H	☐ :	**Prélèvements:** sang voie artérielle	*(2.7 ml)*
☐ :	☐ :	sang voie veineuse	*(2.7 ml)*
	☐ :	filtrat/dialysat (sortie du filtre)	*(2.7 ml)*
t = 3 H	☐ :	**Prélèvements:** sang voie artérielle	*(2.7 ml)*
☐ :	☐ :	sang voie veineuse	*(2.7 ml)*
	☐ :	filtrat/dialysat (sortie du filtre)	*(2.7 ml)*
		Appareil: Débit sanguin: ☐ ml/min	
		Débit sol. prédilution: ☐ l/h Débit postdilution: ☐ l/h	
		Débit de dialyse: ☐ l/h Prél. patient: ☐ l/h	
		P. du retour sanguin: ☐ mm Hg Débit UF: ☐ l/h	
		P. du filtrat/dialysat: ☐ mm Hg *(évent.hauteur urimètre-filtre x0.75)*	
		Patient: Pression sys/dias: ☐ / ☐ mmHg T°: ☐ °C	
		Fréquence resp: ☐ rpm P.veineuse centrale: ☐ mm Hg	
		Rythme card: ☐ bpm Saturation O2: ☐ % *(pulsoxymétrie)*	
t = 4 H	☐ :	**Prélèvements:** sang voie artérielle	*(2.7 ml)*
☐ :	☐ :	sang voie veineuse	*(2.7 ml)*
	☐ :	filtrat/dialysat (sortie du filtre)	*(2.7 ml)*
NB: éventuellement:			
	☐ :	changement sac d'urine vol. ☐ ml et prélèvement	
	☐ :	changement sac filtrat/dialysat vol.: ☐ ml et prélèvement	
	☐ :	changement de filtre	

Date: ____ Etape: ____ No. de patient dans l'étude: ____

H.théorique	Hréelle	A faire	Remarques (no de renvoi)
t = 6 H	:	**Prélèvements:** sang voie artérielle	*(2.7 ml)*
:	:	sang voie veineuse	*(2.7 ml)*
	:	filtrat/dialysat (sortie du filtre)	*(2.7 ml)*
		Appareil: Débit sanguin: ____ ml/min	
		Débit sol. prédilution: ____ l/h Débit postdilution: ____ l/h	
		Débit de dialyse: ____ l/h Prél. patient: ____ l/h	
		P. du retour sanguin: ____ mm Hg Débit UF: ____ l/h	
		P. du filtrat/dialysat: ____ mm Hg *(évent.hauteur urimètre-filtre x0.75)*	
		Patient: Pression sys/dias: ____ / ____ mmHg T°: ____ °C	
		Fréquence resp: ____ rpm P.veineuse centrale: ____ mm Hg	
		Rythme card: ____ bpm Saturation O2: ____ % *(pulsoxymétrie)*	
t = 12 H	:	**Prélèvements:** sang voie artérielle	*(2.7 ml)*
:	:	sang voie veineuse	*(2.7 ml)*
	:	filtrat/dialysat (sortie du filtre)	*(2.7 ml)*
		Appareil: Débit sanguin: ____ ml/min	
		Débit sol. prédilution: ____ l/h Débit postdilution: ____ l/h	
		Débit de dialyse: ____ l/h Prél. patient: ____ l/h	
		P. du retour sanguin: ____ mm Hg Débit UF: ____ l/h	
		P. du filtrat/dialysat: ____ mm Hg *(évent.hauteur urimètre-filtre x0.75)*	
		Patient: Pression sys/dias: ____ / ____ mmHg T°: ____ °C	
		Fréquence resp: ____ rpm P.veineuse centrale: ____ mm Hg	
		Rythme card: ____ bpm Saturation O2: ____ % *(pulsoxymétrie)*	
t = 24 H	:	**Prélèvements:** sang voie artérielle	*(2.7 ml)*
:	:	sang voie veineuse	*(2.7 ml)*
	:	filtrat/dialysat (sortie du filtre)	*(2.7 ml)*
		Appareil: Débit sanguin: ____ ml/min	
		Débit sol. prédilution: ____ l/h Débit postdilution: ____ l/h	
		Débit de dialyse: ____ l/h Prél. patient: ____ l/h	
		P. du retour sanguin: ____ mm Hg Débit UF: ____ l/h	
		P. du filtrat/dialysat: ____ mm Hg *(évent.hauteur urimètre-filtre x0.75)*	
		Patient: Pression sys/dias: ____ / ____ mmHg T°: ____ °C	
		Fréquence resp: ____ rpm P.veineuse centrale: ____ mm Hg	
		Rythme card: ____ bpm Saturation O2: ____ % *(pulsoxymétrie)*	
t = 36 H	:	**Prélèvements:** sang voie artérielle	*(2.7 ml)*
:	:	sang voie veineuse	*(2.7 ml)*
	:	filtrat/dialysat (sortie du filtre)	*(2.7 ml)*
		Appareil: Débit sanguin: ____ ml/min	
		Débit sol. prédilution: ____ l/h Débit postdilution: ____ l/h	
		Débit de dialyse: ____ l/h Prél. patient: ____ l/h	
		P. du retour sanguin: ____ mm Hg Débit UF: ____ l/h	
		P. du filtrat/dialysat: ____ mm Hg *(évent.hauteur urimètre-filtre x0.75)*	
		Patient: Pression sys/dias: ____ / ____ mmHg T°: ____ °C	
		Fréquence resp: ____ rpm P.veineuse centrale: ____ mm Hg	
		Rythme card: ____ bpm Saturation O2: ____ % *(pulsoxymétrie)*	

NB: éventuellement:

:	changement sac d'urine vol. ____ ml et prélèvement
:	changement sac filtrat/dialysat vol.: ____ ml et prélèvement
:	changement de filtre

Date: ☐ **Etape:** ☐ **No. de patient dans l'étude:** ☐

H.théorique	Hréelle	A faire	Remarques (no de renvoi)
t = 48 H	:	**Prélèvements:** sang voie artérielle + 1 tube urée/créatinine	*(2.7 + 1.5 ml)*
:	:	sang voie veineuse	*(2.7 ml)*
	:	filtrat/dialysat (sortie du filtre)	*(2.7 ml)*
	:	**Changement:** récipient filtat/dialysat	
	:	sac urine	
		Mesures: Volume filtrat/dialysat total: ☐ l	
		Volume urinaire total (si sonde): ☐ l	
		Appareil: Débit sanguin: ☐ ml/min	
		Débit sol. prédilution: ☐ l/h Débit postdilution: ☐ l/h	
		Débit de dialyse: ☐ l/h Prél. patient: ☐ l/h	
		P. du retour sanguin: ☐ mm Hg Débit UF: ☐ l/h	
		P. du filtrat/dialysat: ☐ mm Hg *(évent.hauteur urimètre-filtre x0.75)*	
		Patient: Pression sys/dias: ☐ / ☐ mmHg T°: ☐ °C	
		Fréquence resp: ☐ rpm P.veineuse centrale: ☐ mm Hg	
		Rythme card: ☐ bpm Saturation O2: ☐ % *(pulsoxymétrie)*	
	:	**Bilan final:** niveaux apports (sans pré/postdilution)fin observation: ☐ l	
		niveau sol. prédilution fin observation: ☐ l (détail au verso)	
		niveau sol. postdilution fin observation: ☐ l	
		Prélèvement: filtrat/dialysat total *(2.5 ml)*	
		(récolté durant la période d'observation)	
		Apports cumulés durant la période d'observation:	
		Apports (sans compensation): ☐ l	
		Sol. prédilution: ☐ l	
		Sol. postdilution: ☐ l	

Ne pas oublier pointage des valeurs: ventilation, Swan, labo, gazo, et médicaments

Remarques: