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ACICLOVIR-INDUCED NEUROPSYCHIATRIC SYMPTOMS- A CLINICAL PHARMACOLOGY STUDY

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“Music was my first love”
John Miles (1949-)

“It’s always nice with a hit“
Marcus Ingman (1959-)

To the memory of my father

ABSTRACT

Aciclovir (ACV) and its prodrug valacyclovir (VACV) are used to treat infections caused by herpes simplex virus (HSV) and varicella zoster virus (VZV), and as prophylaxis against cytomegalovirus (CMV) infections in immunocompromised patients. Treatment with ACV has decreased the mortality in herpes simplex encephalitis (HSE) from about 70 % to less than 30 %, and has also reduced the morbidity. ACV is excreted mainly by the kidneys, while a minor proportion is metabolized to 9-carboxymethoxymethylguanine (CMMG) and 8-hydroxy-ACV. It has been found that CMMG concentrations increase if renal function is decreasing. ACV is considered to have low toxicity and benign side effects. However, increasing serum creatinine and acute renal failure have been reported, and in rare cases aciclovir-induced neuropsychiatric symptoms (AINS) have developed. The mechanism behind AINS is unknown.

The aim of this thesis was to investigate if there is a correlation between increased serum and cerebrospinal fluid (CSF) concentrations of CMMG, and the development of AINS. In paper I we studied the ACV and CMMG serum concentrations in 44 asymptomatic patients and 49 patients with AINS. The CMMG concentration was significantly higher in AINS patients than in the asymptomatic group ($p<0.001$). A ROC analysis showed that a cut-off value of 11 µmol/L had the highest sensitivity and specificity to predict AINS compared to other predictors, such as ACV exposure, ACV concentrations and renal function tests.

The hypothesis that CMMG is directly involved in AINS implies that it is present in the CNS. In paper II we did find measurable concentrations of CMMG in CSF, but only in subjects with AINS. Asymptomatic control subjects had CSF CMMG levels below the limit of detection.

Paper III is a description of two cases developing Cotard's syndrome- a delusion of being dead- as a result of ACV treatment. Both patients had high serum concentrations of ACV and CMMG.

In paper IV the signs and symptoms pattern of AINS were analyzed in a larger group of patients. We retrieved data from published case reports, ADR cases reported to the Swedish Medical Products Agency and from our own Therapeutic Drug Monitoring database, in all 275 cases. We also compared AINS with signs and symptoms from herpes encephalitis (HSE). The study confirmed that altered level of consciousness, confusion, and hallucinations were the most frequent clinical characteristics of AINS, while HSE patients presented with high fever, altered level of consciousness, focal neurological signs, and headache.

Paper V is a single-dose, open-label pharmacokinetic crossover study with one intravenous part and one oral part. We found that CMMG levels in hemodialysis patients were more than 10 times higher than in healthy volunteers. Subjects with normal and moderately impaired renal function had CMMG levels close to, or below, measurable concentrations.

The results of this thesis support the hypothesis that CMMG is a useful predictor of AINS and that it may be mechanistically involved. Symptoms of AINS cannot always be reliably discriminated from symptoms of HSE, and vice versa. Determination of serum and CSF CMMG concentrations may be a useful tool to diagnose AINS and when distinguishing between AINS and HSE. Dose regimens aimed to minimize CMMG exposure can be developed based on the PK results.

LIST OF PUBLICATIONS

This thesis is based on the following publications and manuscripts, referred to in the text by their Roman numerals:

I. **Helldén A**, Odar-Cederlöf I, Diener P, Barkholt L, Medin C, Svensson J-O, Säwe J, Ståhle L. High serum concentrations of the acyclovir main metabolite 9-carboxymethoxymethylguanine in renal failure patients with acyclovir-related neuropsychiatric side effects.
Nephrol Dial Transplant. 2003 Jun;18(6):1135-41

II. **Helldén A**, Lycke J, Vander T, Svensson JO, Odar-Cederlöf I, Ståhle L. The aciclovir metabolite CMMG is detectable in the CSF of subjects with neuropsychiatric symptoms during aciclovir and valaciclovir treatment.
J Antimicrob Chemother. 2006 May;57(5):945-9. Epub 2006 Mar 15.

III. **Helldén A**, Odar-Cederlöf I, Larsson KA, Fehrman-Ekholm I, Lindén, T. Death delusion.
BMJ. 2007 Dec 22;335(7633):1305

IV. **Helldén** A. Lindén T, Odar-Cederlöf I, Bergman U, Ståhle L. Clinical characteristics of aciclovir-induced neuropsychiatric symptoms in 275 patients. (In manuscript)

V. **Helldén A**, Odar-Cederlöf I, Pohanka A, Ståhle L. Single-dose pharmacokinetics of acyclovir and its principal metabolite 9-carboxymethoxymethylguainine (CMMG) in subjects with normal and impaired renal function and in hemodialysis patients**.** (In manuscript)

Related work:

Mahad D, Jarvis J, Chinnery PF, Mitra D, Gholkar A, **Helldén A**. Aciclovir induced posterior leucoencephalopathy. J Neurol Neurosurg Psychiatry. 2005 Sep;76(9):1308-9.

Helldén A, Bergman U, Dwyer, R, Medin C, Molanaei H, Ståhle L. Thylén L, Odar-Cederlöf I. Risk för CNS-biverkningar av aciclovir och valaciclovir- se upp med njurfunktionen! [Risk of CNS adverse effects of aciclovir and valaciclovir. Watch the renal function in treatment of herpes simplex and herpes zoster] Läkartidningen, förhandspublicerad på nätet 2007-05-10, i tryckt version 2007;24-25,1916-20.

van Berlo-van de Laar IRF. Goet ER, **Helldén A**, Sluiter HE. [Adverse effect of valaciclovir in disturbed kidney function) Neurotoxiciteit door valaciclovir gebruik bij patiënten met nierfunctiestoornissen: wanneer meten van aciclovir en 9-carboxymethoxymethylguanine serumconcentraties? Tijdschr. voor Geneeskunde. Ned Tijdschr Geneeskd. 2009 Apr 11;153(15):706-9

CONTENTS

LIST OF ABBREVIATIONS

8-OH-ACV	8-hydroxy-9-(2-hydroxyethoxymethyl)guanine
ACV	Aciclovir
ACV-TP	Aciclovir triphosphate
ADH	Alcohol Dehydrogenase
ADR	Adverse Drug Reaction
ALDH	Aldehyde Dehydrogenase
AUC	Area Under the Curve
CKD	Chronic Kidney Disease
CL	Clearance
Cmax	Maximum concentration
CNS	Central Nervous System
CrCL	Creatinine Clearance
CSF	Cerebrospinal Fluid
CMMG	9-Carboxymethoxymethylguanine
ELISA	Enzyme-linked immunosorbent assay
ESRD	End-Stage Renal Disease
IC50	Mean 50% inhibitory concentration
GCV	Ganciclovir
GFR	Glomerular Filtration Rate
HD	Hemodialysis
HPLC	High Pressure Liquid Chromatography
HSV	Herpes Simplex Virus
i.v.	Intravenous
LC-MS	Liquid Chromatograph-Mass Spectrometry
LOQ	Limit of Quantification
MDRD	Modification of Diet in Renal Disease
MRI	Magnetic Resonance Imaging
PCR	Polymerase Chain Reaction
PD	Peritoneal Dialysis
PDR	Physicians Desk Reference (FASS in Sweden)
PK	Pharmacokinetics
qid	“quater in die” (four times a day)
RIA	Radioimmunoassay
SWEDIS	Swedish Drug Information System
t½	Half-life
tds	“ter die sumendum” (three times a day)
TDM	Therapeutic Drug Monitoring
VACV	Valaciclovir
VZV	Varicella Zoster Virus

1 INTRODUCTION

1.1 GENERAL INTRODUCTION

The antiviral drug aciclovir[1] (ACV) is used to treat infections caused by alphaherpes viruses, i.e. herpes simplex virus (HSV) and varicella (herpes) zoster (VZV), and the betavirus cytomegalovirus (CMV). It is the drug of choice for treatment of neonatal herpes simplex virus (HSV) infections, herpes simplex encephalitis (HSE), and HSV and VZV infections in immunocompromised patients. Valaciclovir (VACV), the L-valyl ester of ACV is also used as prophylaxis against CMV infections in solid organ recipients and bone marrow transplanted patients.

ACV is considered to have low toxicity compared to other antiviral drugs with effect against these viruses, such as ganciclovir (GCV), foscarnet and cidofovir (CDV) [1]. Common side effects include nausea, vomiting, diarrhoea, and headache [2]. Other side effects such as increasing creatinine and acute renal failure have been reported in dehydrated patients or in cases where ACV has been given too fast intravenously (i.v.) [3,4].

In spite of its low toxicity, aciclovir-induced adverse drug reactions from the central nervous system (CNS) have been reported. These symptoms, in general designated as neurotoxic or neuropsychiatric, have caused suffering for patients and costs for the health system. The mechanism behind CNS-effects is unknown. High ACV concentrations have been reported [5], while others have shown normal levels [6]. Aciclovir-induced neuropsychiatric symptoms (AINS) have in several cases been difficult to distinguish from CNS symptoms caused by the infection it is treating (confounding by indication). On suspicion of herpes or zoster encephalitis the doses have been increased and changed from oral to intravenous administered drug, which in turn has worsened the condition [7,8].

In this thesis I put forward a hypothesis that increased concentrations of the ACV main metabolite 9-carboxymethoxymethylguanine (CMMG) is a marker for and possibly also a cause of AINS. I will also suggest that Therapeutic Drug Monitoring (TDM) of CMMG may be a simple and fast diagnostic tool to distinguish between AINS and symptoms of CNS infections.

The thesis will present various aspects of the discipline Clinical Pharmacology. My work starts with a case report of an adverse drug reaction and demonstrates the usefulness of a TDM database, continues with clinical pharmacology and epidemiology studies describing the pattern of AINS and ends with a pharmacokinetic (PK) study.

[1] ACV is sometimes spelled acyclovir and sometimes aciclovir. The reason for this discrepancy is unclear to me, but *acyclo*vir is referring to "Acyclovir, an acyclic purine nucleoside analog [32] and is in use in American English, while aciclovir is usually used in British English.

1.2 THE INDEX CASE

In April 1994, one of my diabetic patients on peritoneal dialysis (PD) training presented with blisters on his chest. Herpes simplex (HS) was suspected but, as the patient had been a predialysis patient for some time and was likely to have an impaired immune system, shingles, or varicella zoster, could not be ruled out. Shingles was suspected despite the fact that the blisters appeared on both sides of the chest, while they usually appear unilateral. Aciclovir treatment was planned to be initiated with a dose adapted for the renal function (oral ACV 800 mg x 2) according to the dose recommendation in the Swedish PDR (Physicians Desk Reference).

Earlier experiences among colleagues at the Department of Nephrology revealed that CNS ADRs had occurred in some patients with end-stage renal disease (ESRD), but the relation to aciclovir dose or concentration was not clear. I therefore decided to contact the Zovirax® (ACV) product specialist at GlaxoWellcome to get advice regarding dose recommendations for PD patients. In a letter that I received the day after the first telephone discussion, I was advised to follow the recommendations in the PDR to avoid treatment failure. I was also told that the Department of Clinical Pharmacology at Huddinge University Hospital, Stockholm, had a method for analysing ACV concentrations. I decided to give the recommended dose, but also to measure the ACV concentrations during the 5 day treatment period, both in serum and in PD effluent. The patient agreed on visiting the department every morning during that period, bringing his PD bags from the previous evening and night. A swab was used for diagnostic tests of the blisters. Concomitant treatment was ciprofloxacin and vancomycin, instituted some weeks before the event due to a skin infection. He was also treated with other drugs commonly seen in dialysis patients, and there had been no changes in the treatment regiment.

The patient took the first 800 mg ACV dose in the afternoon. The next day he came to the clinic at noon collecting blood and leaving PD-effluent. He also administered the second 800 mg ACV dose before leaving the department. The patient called me that same evening. He told me that about 3 hours after the second ACV dose he had begun to feel strange and oddly depersonalized. He wondered if the ACV treatment might be causing the symptoms. I decided to withdraw ciprofloxacin, which is known to cause CNS adverse reactions, but to continue with ACV.

The patient did not turn up to collect blood and leave his PD bags the next morning. I called him at noon, and a tired patient answered saying: “I´ve had the worst night of my life! I’ve been awake all night, afraid to fall asleep, because whenever I closed my eyes I saw snakes and flies and multiple TV-sets. I’m aching all over and my hands are shaking! Even though I’ve never tried illegal drugs, I’m sure you can compare this horrifying experience with a bad trip on LSD!”

The patient finally came to the department, but not until 4 o'clock in the afternoon. He had had no further hallucinations, but his hands were still shaking and he was still very tired. He said that his wife had taken the ACV pills and flushed them down the toilet. I made a physical examination of the patient; it was negative except for the tremor and a slight increase in blood pressure. Blood and PD effluent samples from the patient were collected. I explained that he had probably experienced a severe ADR and that I wanted to investigate the cause.

He continued having tremor and muscle pain for another five or six days, but did not experience any other symptoms. ACV concentrations in serum were not remarkably high and seemed not to provide an explanation to the course of events (Figure 1).

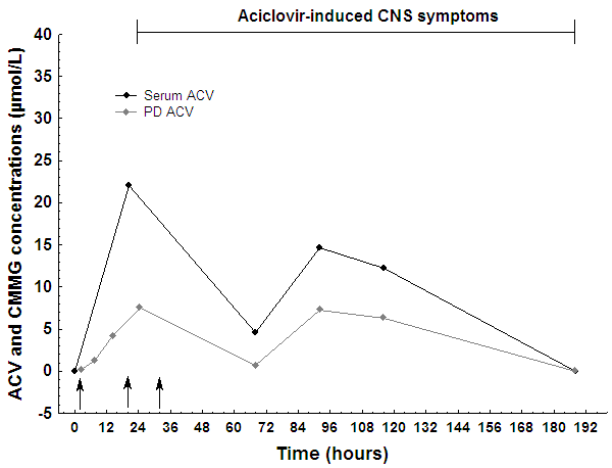

Figure 1. ACV serum and PD-effluent concentrations collected from the index patient. Note that the serum ACV peak concentration was increased, but not remarkably high. When dosing with ACV 800 mg x 5 ACV concentrations ranging from min-max 3.5-6.9 µmol/L has been reported in patients with normal renal function. Each arrow represents an oral ACV 800 mg dose. IC_{50} for effect against HSV1 and HSV2, VZV, and CMV have been shown to be 0.3 µmol/L, 3.0 µmol/L, and > 30 µmol/L, respectively [9].

Ciprofloxacin and vancomycin drug levels were also measured, but were within therapeutic range. The result from the blood test was normal, except for a slight increase in CRP. ACV seemed to be the sole culprit, but as ACV levels were increased, though not abnormally high, I excluded it from the list of suspected drugs. The key to the answer was yet to be found.

Approximately one year later, after having done literature research, I decided to once more take contact with the Department of Clinical Pharmacology. Supported by my senior consultant, Dr Anders Tranæus, I asked if they could find any ACV metabolites in the serum collected from my patient. Their answer was that they did find increased concentrations of the ACV main metabolite 9-carboxymethoxymethylguanine (CMMG) (Figure 2). The CMMG increase correlated in time with the patients neuropsychiatric symptoms. In addition, I was told that they had seen increased concentrations of CMMG also in other patients

reported to have suspected CNS side effects, but that the finding had not yet been explored.

Was my patient the only case or could I find other AINS patients with increased CMMG concentrations? To find an answer to this question I investigated an ACV-treated group of ESRD patients with and without suspected AINS. ACV, but not CMMG, serum concentrations had earlier been measured in these patients, making a blinded comparison possible. The result was promising! AINS patients had high CMMG concentrations while asymptomatic patients had low concentrations. The moment I received these results marked the beginning of my career as a scientist!

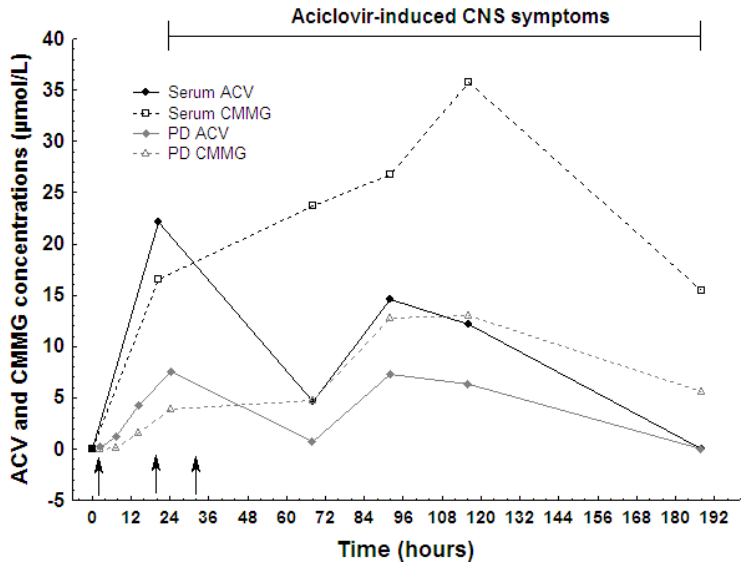

Figure 2. This is figure 1 with addition of CMMG concentrations. When serum and PD-effluent concentrations were analyzed, they initially showed a steep increase in ACV and CMMG levels, corresponding to the initial neuropsychiatric symptoms. Still seven days after the first ACV dose CMMG levels remained increased and the patient had persistent muscle pain and tremor. Each arrow represents an oral ACV 800 mg dose.

1.3 HERPES SIMPLEX

The word "*herpes"* derives from ancient Greek and means to "*creep*" or "*crawl*". Herpes diseases have been known to man for at least three millennia. Both herpes simplex (HSV) and varicella zoster (VZV) viruses are ubiquitous human alphaherpes double-stranded DNA-viruses. HSV is divided into two subgroups. HSV1 is usually transmitted during early childhood and seropositivity has reached 80-90 % worldwide. HSV2 is still the most common cause of sexually transmitted HSV infections. HSV is transmitted via mucosal surface or damaged skin, HSV1 usually via the orolabial route and HSV2 normally through sexual contact. However, HSV1 can also cause genital herpes and HSV2 can also cause herpes labialis [10].

1.3.1 Latency and reactivation

A primary HSV infection may be asymptomatic, but is clinically recognised by the presence of typical herpes vesicles. After entering the body the virus is transported via the fast axonal retrograde transport to the soma of neuron, where it can rest for a long period of time. Sunbathing, having a cold or being exposed to stress may activate the virus, but the mechanism behind reactivation is still unknown. After reactivation the virus is transported into the nerve axon and back to the skin or genitals, where it causes painful blisters and ulcers. Recurrent HSV infections are usually mild, but may be traumatic and embarrassing. HSV can also cause more severe diseases such as neonatal HSV2 and herpes encephalitis (HSE) [10].

1.3.2 Neonatal HSV2

Neonatal HSV2 is a disease with high mortality and morbidity despite aciclovir treatment. The virus is transferred to a child during birth. ACV prophylaxis during pregnancy has been discussed, but since the drug may cause neutropenia and nephrotoxicity ACV prophylaxis has not been supported [10]. In neonates the standard dose of ACV is 60 mg/kg/day.

1.3.3 Herpes encephalitis

Herpes encephalitis (HSE) is a devastating infection in the CNS and the primary cause of sporadic fatal encephalitis in the United States [11,12]. Before effective antiviral treatment was available approximately 70 % of all infected died and the remaining 25-30 % suffered from severe sequelae such as memory impairment or seizures. Intranuclear inclusion bodies with HSV infection were demonstrated for the first time in the brain of a neonate in 1941, and the virus was subsequently isolated from the patient's brain tissue [13]. HSE can occur in all age groups, but has a bimodal distribution. Roughly 30 % of all infected are below 20 years of age and 50 % are over 50 [11,14]. The annual incidence in USA has been estimated to be 2000 cases [15]. In Sweden, the annual incidence is 2.2 per million inhabitants, corresponding to 20 cases per year [16]. Most infections (90 %) are due to HSV1 [17].

1.3.4 Clinical features of HSE

Distinguishing between viral infection and AINS is a diagnostic and therapeutic challenge. Since ACV should be instituted without delay in every suspected case of HSE, knowledge of the clinical features of HSE is important early in the diagnostic

procedure, when still waiting for results from herpes PCR and neuroimaging. Several retrospective and prospective studies on the clinical features of HSE have been reported and some of them are presented in Table 1. The prodromal symptoms may include influenza-like illness, high fever, and headache. Later clouding of consciousness and confusion may develop [18-21] (Table 1).

Table 1. Clinical features of herpes simplex encephalitis (HSE)

Study	**Kennedy**	**Sköldenberg**	**Whitley**	**McGrath**
No of patients	46	53	28	42
Symptoms	Per cent of investigated patients			
Influenza-like symptoms	48 %			
Headache, confusion and alteration of the level of consciousness	52 %			
Confusion/Disorientation				67 %
Altered consciousness		100 %	96 %	90 %
Lethargy		*(57 %)*	*(39 %)*	
Semicoma		*(30 %)*	*(25 %)*	
Deep coma	35 %	*(13 %)*	*(36 %)*	
Fever	100 %	100 %	96 %	98 %
Personality changes		87 %	95 %	24 %
Headache		74 %	82 %	74 %
Meningism	65 %			55 %
Drowsiness	65 %			45 %
Muteness, dysphasia, or aphasia	46 %	36 %	88 %	28 %
Raised intracranial pressure	33 %			
Focal neurological signs	89 %			
Seizures	61 %	62 %	57 %	50 %
- Focal	*(50 %)*		*(43 %)*	
-Generalised	*(9 %)*		*(14 %)*	
- Focal and generalized	*(2 %)*			
Vomiting		38 %	46 %	
Disorientation		57 %	93 %	
Hemiparesis		40 %	43 %	33 %
Lethargy				24 %
Memory loss			18 %	
Photophobia			4 %	
Autonomic-nervous system dysfunction			75 %	
Ataxia			67 %	

1.3.5 Management of HSE

HSV encephalitis is a medical emergency. Immediate and accurate diagnosis with the use of lumbar puncture, neuroimaging, and PCR is crucial. ACV is the first line treatment and should be initiated in every suspected case. Normal dosing is 10 mg/kg intravenously every 8 h (30 mg/kg/day) for 14 days, 21 days for immunocompromised patients. The dose should be adapted to renal function [15,22,23]. Early treatment is crucial to prevent severe sequelae and to decrease mortality.

ACV is nowadays instituted in nearly all cases with suspected encephalitis, even if the diagnosis is unclear. There are mainly two drawbacks to this procedure: firstly, it

creates a feeling of safety and might prolong the time to actual diagnosis, secondly, it may cause nephrotoxicity and AINS [15].

1.4 VARICELLA AND HERPES ZOSTER

Varicella (chickenpox) is caused by the varicella zoster virus (from latin "*variola*"= little pox and the Greek word "*zoster"*= girdle) and affects mainly children and adolescents. The seroconversion rate is about 95 % in children in United States [24]. The virus may be latent in the cranial and dorsal root ganglia of sensory nerves for many years, but can reactivate to herpes zoster or shingles (from Latin "*cingulum*"= belt or girdle). Shingles is also called "Zona", "Gürtelrose", or "Hells fire".

1.4.1 Age-related reactivation

Reactivation may occur in any age group, but the risk increases with age. A study showed an annual incidence of 200 cases per 100 000 population at the age of 30, but increased to 800-1200/100 000 at the age of 80 [25]. Reactivation occurs in the ganglia, the virus travels along the sensory nerve into the skin and causes shingles. Shingles affects the dermatom which the ganglia innervate. Shingles are painful and can cause postherpetic pain, which can persist for months [26]. As with HSV, the reason why the virus reactivates is still not known, but stress, diseases such as HIV or cancer, or treatment with immunosuppressants, increases the risk.

1.4.2 Herpes zoster encephalitis (HZE)

An increasing body of evidence indicates that HZE is more common than earlier believed. In a Finnish collaborative study of 3231 patients, VZV was the main agent associated with encephalitis, followed by HSV and enteroviruses. Improved diagnostic methods, especially PCR, can be a factor explaining the increase [27]. In a later study the same group found that 44 % of 174 patients with PCR-proven VZV had no cutaneous symptoms at all, 34 % had shingles and 22 % had chickenpox. Of interest for this thesis is the finding that HZE clinical symptoms were similar to AINS [28].

1.4.3 Treatment of Herpes Zoster (shingles)

The recommended doses for treatment of shingles are usually higher than for treatment of HSV. The doses for patients with normal renal function are 1000 mg x 3 or 800 mg x 5. For patients with renal impairment (GFR 10-30 mL/min) the recommended dose is VACV 1000 mg x 1 or oral ACV 800 mg x 3. For patients with ESRD the doses are VACV 500 mg x 1 or oral ACV 800 mg x 2, but may probably be too high for vulnerable patients.

1.5 ACICLOVIR

The high mortality and morbidity in HSE prompted studies on antiviral drug candidates. Several attempts were made to find a drug that was easy to administer, nontoxic, and with a favourable pharmacokinetic profile. Compounds such as trifluorothymidine, idoxuridine (5-iodo-2'-deoxyuridine) and ara-C (1-beta-D-arabinofuranosyl cytosine) were tested, but found to be too toxic (ara-C is now a frequently used chemotherapeutical- cytarabine). However, vidarabine (adenine arabinoside) was reported to be effective against both HSE and HZE, but could only be administered intravenously [18]. An extensive search for a more effective and less toxic compound was begun at Burroughs Wellcome in the early 1960s [29]. Aciclovir was discovered in 1974 [30] and after intense preclinical testing the drug entered clinical development in 1977. The results from phase II studies brought topical, intravenous and oral formulations to the market, starting with the topical form in 1982 [29].

1.5.1 Aciclovir mode of action

When ACV enters a virusinfected cell the viral thymidine kinase phosphorylates ACV to ACV-monophosphate, which subsequently is phosphorylated by cellular enzymes to the active substance ACV-triphosphate (ACV-TP) [31,32]. ACV inhibits the synthesis of viral DNA by eventually incorporating ACV-monophosphate into the viral DNA in an irreversible process. ACV also inhibits the viral DNA polymerase. The human thymidine kinase has a much lower affinity for ACV than the viral kinase. Uninfected cells will thus only form small amounts of ACV-TP. Other drugs, such as famciclovir (FCV), brivudin (BVDU) and ganciclovir (GCV), use the same viral mechanism [1]. An *in vitro*-study showed that it takes roughly 6-8 hrs to reach maximum intracellular amounts of ACV-TP in HSV-infected Vero cells [33]. Yet another *in vitro*-study showed that ACV-TP had an intracellular half-life of 1-2 hours [34].

1.5.2 ACV pharmacokinetics

1.5.2.1 General pharmacokinetics

ACV is a small molecule with a molecular weight of 225 D. The protein binding is around 10-20 %. Oral ACV has a low and dose dependant bioavailability (F), ranging from 9 % to 23 %. Steady-state peak plasma[2] concentrations after 200, 400 and 600 mg of ACV every 4 hrs were 2.22 ± 0.0, 5.3 ± 1.3 and 5.8 ± 1.3 µmol/L, respectively. Total oral body clearance ($ACV_{CL\ tot}$) was 1884 ± 464, 1765 ± 652 and 2217 ± 1217 mL/min/1.73m^2, respectively. Half-life was 3.3 ± 0.4 hrs [35]. The major route of elimination is by glomerular filtration and tubular secretion [36]. The development of VACV, the L-valyl ester of ACV, improved the oral ACV bioavailability from 9-23 % to 50-70 %. Early studies found that over 95 % of ACV was excreted unchanged in the urine in healthy subjects. A small fraction was excreted as the main metabolite 9-carboxymethoxymethylguanine (CMMG) and less than 0.2 % was excreted as 8-hydroxy-9-(2-hydroxyethoxymethyl)guanine (8-OH-ACV) [37]. A later VACV study reported that the majority of recovered ACV in urine was acyclovir (84.3 ± 4.7 %),

[2] ACV concentrations were analyzed in plasma in early studies, while serum was used at our laboratory.

CMMG (13.8 ± 4.2 %) and 8-OH-ACV (1.5 ± 1.1 %) [38]. A VACV dose of 1000 mg every 8 h produced an exposure (AUC) similar to an i.v. ACV dose of 5 mg/kg tds [28].

1.5.2.2 ACV metabolism

In vitro studies suggested that ACV was metabolised to CMMG by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), the two main enzymes metabolizing ethanol. Aldehyde oxidase was responsible for the formation of 8-OH-ACV [39]. Aciclovir aldehyde, the proposed intermediate formed by ADH, has been synthesized and is readily convertible back to ACV, as well as to CMMG [40]. Early studies showed that a greater amount of ACV is excreted as CMMG when renal function is declining [35]. Both ADH and ALDH enzymes are polymorphic and may thus have different metabolic capacity [41].

1.5.2.3 Intravenous administration

The pharmacokinetics of i.v. ACV can be described by a 2-compartment model [35, 42], although a 1-compartment model has also been used [43]. Total ACV clearance after i.v. infusion ranged from 117 to 396 mL/min/1.73m^2 [44].

1.5.2.4 Impaired renal function

Total ACV clearance decreases with age, probably due to the physiological decrease of renal function in elderly patients. A linear relation between total ACV clearance and CrCL has been reported: total ACV clearance = 28.7 + 3.37*CrCL. The terminal plasma half-life in ESRD patients ranged from 13-29 hours, (mean ± SD) 19.5 ± 5.9 hrs. A single hemodialysis reduced the plasma ACV concentrations by 60 % [35].

1.5.2.5 ACV in CSF

The concentration of ACV in CSF has been reported to be about 50 % of the ACV serum concentrations, irrespective of route of administration [36].

1.5.3 ACV side effects

The neuropsychiatric side effects reported are discussed in section 1.8. Other ACV-induced side-effects, such as bone-marrow toxicity [45] and nephrotoxicity, have also been reported [46-50]. The mechanism behind the development of acute renal failure (ARF) is still under debate. It has been suggested that rapid infusion of ACV may cause ARF due to precipitation of ACV crystals in the tubular cells [4, 51-56]. Gunness et al suggested in an *in vitro* study that acyclovir may induce direct insult to renal proximal tubular cells [57].

1.5.4 Possible toxicity of CMMG

A thorough search on CMMG toxicity in relevant databases, such as PubMed, Embase and similar sources, gave no information on toxicity. This was confirmed by GlaxoSmithKline, stating that pre-clinical studies conducted during the development of ACV did not focus on CMMG. Accordingly, the company does not have pre-clinical data relating to CMMG and the occurrence of neurological events (Steve Weller, GlaxoSmithKline, personal communication, 2010)

1.5.5 HSV and VZV sensitivity (IC_{50}) to ACV

When dosing ACV it is important to know the antiviral activity of the drug, especially as different types of herpesviruses will show different susceptibility. The ACV antiviral activity has been measured by *in vitro* systems showing that HSV1 and HSV2 had low (IC_{50}) values, approximately < 0.3-0.3 μM, VZV about 3.0 μM and CMV >30 μM [9]. These *in vitro* values depend on the particular *in vitro* system used. It therefore cannot be taken as a predictive measurement of clinical efficacy but can give a guide to relative efficacy.

1.6 THERAPEUTIC DRUG MONITORING (TDM)

One definition of TDM is "The regular measurement of serum levels of drugs requiring close 'titration' of doses in order to ensure that there are sufficient levels in the blood to be therapeutically effective, while avoiding potentially toxic excess; drug concentration *in vivo* is a function of multiple factors" [58]. Some of these factors are conditions that may affect drug absorption, metabolism or renal excretion and drug-drug interactions (DDIs), like the previously reported interaction between fluconazole and phenytoin [59]. TDM is important for measuring concentrations in drugs with a narrow therapeutic range (e.g. phenytoin), to avoid toxicity or subtherapeutic concentrations, and to measure compliance.

1.7 DRUG ANALYSIS

The high-pressure liquid chromatography (HPLC) method, published in 1977 in the classic paper by Gertrude Elion *et al*, was the first method to measure ACV and metabolites in blood, CSF and urine [31]. Several other methods have been published [60-63]. To our knowledge there is only one documented instance in which CMMG has been found in human plasma [64].

1.7.1 Presently used HPLC assay and determination of CMMG

In 1993-94 a prospective study was carried out of high-dose ACV prophylaxis against CMV infections in liver recipients [65]. As a part of this project an assay to analyze ACV was developed in order to provide rapid analysis results. The method, a reversed-phase solid-phase extraction and HPLC, had the advantage that it could separate CMMG from ACV (Figure 3) [66]. This separation was earlier only possible using the radioimmunoassay method. The assay became available for clinical use in 1991.

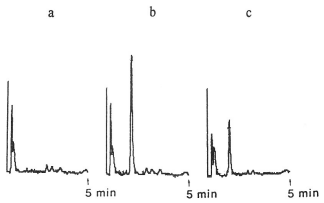

Fig. 2. Chromatograms of blank serum (a) and of serum spiked with 2 μmol/l ACV (b) and 2 μmol/l CMMG (c). Recorder attenuation=2.

Figure 3. The chromatography shows separation of CMMG from ACV [66] *[Jan-Olof Svensson, Lisbeth Barkholt, Juliette Säwe. Copyright (1997), with permission]*

1.8 ACICLOVIR-INDUCED NEUROPSYCHIATRIC SYMPTOMS (AINS)

1.8.1 Background

Case reports on CNS adverse reactions appeared in the medical journals soon after ACV had been established on the market in the beginning of the 1980th century [6,45, 48,49,67-72].

Five years after the introduction of ACV, and when it had been used in over 10 million courses of treatment, the results from a comprehensive epidemiology monitoring program was published. The program reported a" remarkably small number of reports of serious experiences and, of these, the very few in which acyclovir was believed by the reporter to be a cause" [2]. Symptoms from the nervous system were the most or the second most common side-effects reported. As an example an inpatient monitoring study performed in 10 hospitals found that 22 % of 739 ACV-treated patients developed neuropsychiatric symptoms compared to 9 % of 190 controls ($p< 0.001$) [2].

Despite the increasing knowledge of AINS, case reports continue to be published [73-80]. Rashiq *et al* defined the clinical characteristics of aciclovir neurotoxicity in a paper published in 1993, including a case series of 35 patients. The authors concluded that AINS is a self-limiting, dose dependent phenomenon, predominantly affecting elderly patients and patients with renal failure. They also found that it is distinguished from viral encephalitis by absence of fever or headache, lack of focal neurological findings and normal cerebrospinal fluid [81].

Similar instances of AINS were reported when VACV was introduced on the market in 1995 [54,82-90]. In a retrospective case series Das *et al* described 25 cases of AINS in 167 renal transplant recipients (15%) due to VACV treatment. Delayed graft function[3] was found to be the main risk factor (Odds ratio 12.1; 95 % CI= 3.4-43.3; $p< 0.001$) [91]. An ADR bulletin from the Australian Government Therapeutic Goods Administration reported 69 events of VACV adverse reactions. AINS such as dizziness, hallucinations, confusion, and delirium were reported [92].

1.8.2 A definition of AINS

Several of the CNS adverse reactions described or referred to in this thesis are described as "*neurotoxic"*. We have chosen to call the aciclovir-induced ADRs "*neuropsychiatric*" because patients suffering from AINS exhibit both neurological and psychiatric symptoms.

1.8.3 Relation between increased ACV concentrations and AINS

The occurrence of transient ACV-induced adverse reactions, such as vomiting and increasing creatinine in high-dosed subjects in a herpes zoster study suggested that there was a dose correlation [93]. Others have reported a correlation between high ACV serum concentrations and AINS [5,7,45,48,75,76,83,94-106]. However, low or normal ACV concentrations have also been reported [6,54,74,78,107-114].

[3] When a transplanted kidney does not function immediately post-transplant and is "sluggish." The patient may require dialysis until the kidney starts to produce urine and the creatinine level decreases.

2 AIMS

The primary aim of this PhD work was to investigate if there is a relation between ACV, the ACV main metabolite 9-carboxymethoxymethylguanine (CMMG), and the development of AINS.

We also wanted to evaluate if determination of ACV and CMMG in serum could serve as a diagnostic tool for AINS, and to study ACV and CMMG pharmacokinetics. The specific aims in each article were to:

Paper I: investigate the relationship between appearance of AINS and serum concentrations of ACV and CMMG, and to compare the sensitivity and specificity of CMMG and ACV as predictors of AINS.

Paper II: investigate if CMMG is present in cerebrospinal fluid, and if so, to compare the concentrations of CMMG and ACV between subjects with AINS and those without symptoms.

Paper III and IV: describe common clinical features of AINS, sometimes with unusual but possibly unique symptomathology, and if possible, differentiate between AINS and herpes encephalitis symptoms.

Paper V: describe ACV and CMMG pharmacokinetics in subjects with varying degrees of renal function.

3 METHODS AND RESULTS

3.1 PATIENTS

Patient data in study I was retrieved from an ACV TDM database at the Department of Clinical Pharmacology, Karolinska University Hospital, Stockholm, Sweden, containing 275 ACV/CMMG samples from 130 patients. The majority of the samples were collected at the Departments of Infectious diseases, Hematology, and Nephrology in Sweden.

In study II, ACV and CMMG CSF concentrations from AINS cases in the ACV TDM database were compared with subjects included in a VACV survey, studying the possible involvement of herpesviruses in the pathogenesis of multiple sclerosis (MS) [115].

In paper III we described two patients with AINS presenting with Cotard's syndrome- a delusion of being dead. One patient was identified during a regular HD session, and data concerning the other patient was extracted from the TDM database and the patient chart.

Study IV included data from three different sources:

1. ACV or VACV- treated patients with AINS reported to SWEDIS, an ADR database at the Swedish Medical Products Agency, including 110 000 causality assessed ADRs in Sweden [116]
2. Data from our TDM database
3. Published AINS cases from 1981 - 2007

Swedish cases, suspected to be identical due to similarities in age, sex, symptoms, and ACV and CMMG concentrations, were included only in the TDM data base. Similarly, our previously published cases [117-119] were not included in published AINS cases.

In study V healthy subjects were recruited from a database at the Clinical Pharmacology Trial Unit (CPTU) at the Department of Clinical Pharmacology, Karolinska University Hospital, Huddinge, Stockholm, Sweden. Subjects with impaired renal function or ESRD were recruited from the Department of Nephrology at the Karolinska University Hospital.

3.2 DATA COLLECTION

Drug concentrations from study I, II and III were collected from our ACV and CMMG TDM database at the Department of Clinical Pharmacology. The database today includes more than 1500 ACV and CMMG serum, urine and CSF samples collected from more than 500 patients.

3.3 RENAL FUNCTION TESTS

In paper I the renal function (GFR) was calculated by the MDRD formula. The formula was developed by the *Modification of Diet in Renal Disease Study Group* including data on 1600 patients with impaired renal function [120]. The only parameters needed for the calculations are S-creatinine, age and correction factors for gender and ethnicity (being or not being Afro American). The unit is expressed as mL/min/1.73 m^2. The MDRD formula was used because weight was lacking in several patients in study I. A more appropriate formula, such as Cockcroft Gaults formula, could thus not be used without a large drop-out. Note that in paper I the dimension for MDRD is erroneously given as mL/min. However, MDRD GFR was used for the GFR calculations in all patient groups. Children's GFR was calculated by Schwartz estimate [121]. The Cockcroft Gault formula, without correction for body surface area, was used in paper II and V.

3.4 TDM ASSAYS

TDM serum and CSF samples collected from patients in paper I-III were analyzed by the HPLC method developed at our department, as presented in paragraph 1.7.1 [66]. In paper V we described a modified assay with increased sensitivity and specificity using liquid chromatography- mass spectrometry (LC-MS). The disadvantage with the previous HPLC method was that it could not separate CMMG from the antiviral drug GCV, which in some situations was used together with ACV. The problem is solved by using the LC-MS method.

3.5 STATISTICAL METHODS

Data on the concentration of ACV and CMMG were analyzed by Student's *t*-test. Doses were compared by Mann-Whitney *U*-test. Descriptive statistics were generally given as means ± standard deviation (SD), but also as means with 95 % confidence intervals. The frequency data were analyzed using χ^2 test. Most of the data were organized using Microsoft® Excel spreadsheet software (Microsoft Corporation, USA), and statistical calculations were mainly performed using Statistica software (Statsoft Inc, Tulsa, OK, USA). Conventional receiver-operating characteristics (ROC)[4] was used in study I [122]. All studies except study V were retrospective.

[4] A statistical test that can be used to compare the diagnostic performance of two or more laboratory or diagnostic tests. The area under an ROC curve is used as an index of diagnostic performance.

3.6 PAPER I

3.6.1 Study design

Study I was a retrospective study, aiming to test the primary hypothesis of a correlation between increased concentrations of CMMG and the development of AINS. The study was also aiming to compare the sensitivity and specificity of CMMG, ACV, and other parameters such as renal function, as predictors of AINS. Cases and controls were retrieved from the TDM database at the Department of Clinical Pharmacology. Sensitivity and specificity for ACV, CMMG, and renal functions were analyzed by a ROC test.

3.6.2 Results

A total of 93 patients were included in the study. Forty-nine patients had suspected AINS and 44 were asymptomatic or had other reasons for CNS symptoms. We found a statistically significant difference in CMMG serum concentrations between the symptomatic and asymptomatic patients ($P<0.001$), supporting the initial hypothesis. Similarly, ACV concentrations also differed significantly between the two groups ($P<0.01$) (Figure 4). The ROC curve for CMMG demonstrated that neuropsychiatric symptoms could be predicted with a sensitivity of 91 % and a specificity of 93 % with the use of a cut-off value of 11 µmol/L of CMMG. For ACV the sensitivity was 49 % and the specificity 93 % using a cut-off value of 15 µmol/L. Seven of the 49 AINS patients (14 %) had CMMG concentrations above 40 µmol/L (range 40-158 µmol/L).

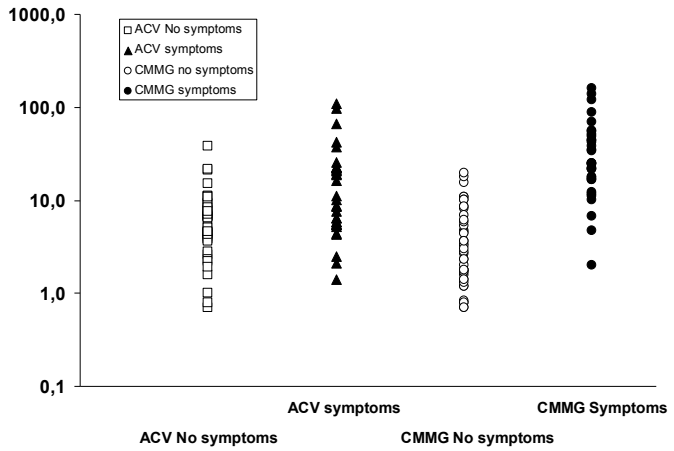

Figure 4 shows the ACV and CMMG serum concentrations in AINS and asymptomatic patients. To convert ACV µmol/L to µg/ml, divide by 4.44 and from CMMG µmol/L to µg/mL, divide by 4.18.

3.7 PAPER II

3.7.1 Study design

To be able to treat herpes encephalitis the penetration of ACV into the brain is crucial. Early studies reported that about 50 % of the ACV plasma concentrations were distributed to CSF [36]. However, these measurements did not include CMMG. The presence of the suspected substance in the brain is likewise a prerequisite for the development of drug-induced neuropsychiatric symptoms. A study of ACV and CMMG levels in CSF in AINS and asymptomatic subjects was therefore conducted. A total of 21 subjects were included in the study, of whom nine had AINS. All AINS patients, except one, had acute or chronic renal failure [123]. Ten of the asymptomatic subjects participated in a pharmacokinetic study on the effects of VACV in multiple sclerosis (MS) [115]. The remaining two subjects had relapsing herpes encephalitis.

3.7.2 Results

CMMG could be detected in CSF only in subjects with AINS, median 1.0 µmol/L (range 0.6 – 7 µmol/L). In asymptomatic subjects the concentration of CMMG was below the limit of quantification (LOQ) (<0.5 µmol/L)($P < 0.001$) (Figure 5). The mean ACV and CMMG serum concentrations in the AINS group were 4.6 and 55.4 times higher, respectively, compared to asymptomatic patients. The ACV CSF/ACV serum ratios in asymptomatic and AINS patients were 0.38 ± 0.34 and 0.88 ± 0.77, respectively ($p=0.061$). The corresponding ratios for CMMG were calculated by setting the CMMG CSF concentrations in the asymptomatic patients to the LOQ value and were 0.69 ± 0.21 and 0.065 ± 0.037, respectively ($p< 0.001$). Both ACV and CMMG showed a linear relation between serum and CSF concentrations.

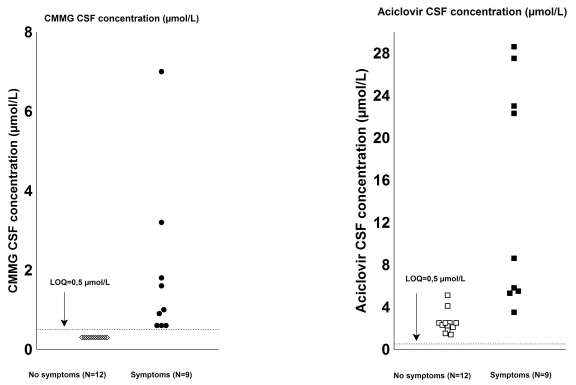

Figure 5. CSF concentrations of 9-carboxymethoxymethylguanine (CMMG) and aciclovir (ACV) from twelve subjects without and nine subjects with neuropsychiatric symptoms. CMMG was detectable in the CSF from symptomatic patients. The twelve subjects without neuropsychiatric symptoms had CMMG CSF concentration below the limit of quantification (LOQ) =0.5 µmol/L. CMMG= 9-carboxy-methoxymethylguanine.

3.8 PAPER III

3.8.1 Study design

Paper III describes two cases of Cotard's syndrome- a rare delusion of being dead [124]. A female patient was on HD due to a failing kidney transplant and a male patient developed acute renal failure after the initiation of VACV treatment. Data concerning the two cases were collected both from the patient charts and in collaboration with the responsible clinicians. ACV and CMMG concentrations came from the TDM database.

3.8.2 Results

Within a few days after initiating VACV treatment both patients presented with confusion and/or depersonalization, symptoms which soon developed into Cotard's syndrome- the belief of being dead. In the female patient the syndrome subsided during a dialysis session. The male patient, who did not receive HD, had CNS symptoms during three days, especially at night (Figure 6). ACV and CMMG concentrations in serum and CSF were high in both patients. Both patients recovered without sequelae.

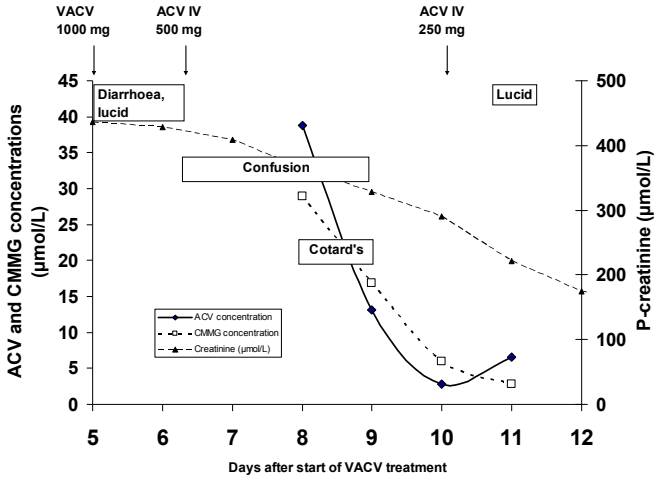

Figure 6. Acyclovir (ACV) (straight line), 9-carboxymethoxymethylguanine (CMMG) (dotted line), and P-creatinine concentrations in relation to neuropsychiatric symptoms and Cotard's syndrome in patient 2.

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3.9 PAPER IV

3.9.1 Study design

In paper IV the signs and symptoms pattern of AINS were studied in a larger group of patients. AINS has earlier been described mainly in case reports including 1-6 patients. Data from SWEDIS, our TDM database and published cases were compiled. Signs and symptoms were collated along with clinical and laboratory findings. In addition, we wanted to evaluate if CNS symptoms in patients with herpes encephalitis could be distinguished from AINS. We therefore studied CNS symptoms found in 81 patients with herpes encephalitis using information from four different HSE studies and compared these with symptoms of AINS [18-21].

3.9.2 Preliminary results

Data from 275 patients with AINS were retrieved; mean age 61.1 ± 20.5 years; 55 % being females. The most frequent neuropsychiatric symptoms were altered level of consciousness (147 patients), confusion (146 patients), hallucinations (95 patients), involuntary movements (79 patients), and impaired coordination (63 patients). The most frequent symptoms, grouped according to preferred terms, are shown in table 2. Renal failure was documented in 215 patients (78 %). ACV was given orally to 188 (70 %) patients and intravenously to 83 (30 %) patients. The main indications were varicella zoster infection in 180 (66 %) patients and herpes simplex infection in 49 (18 %) patients. Almost 60 % of the patients received recommended doses adjusted to renal function.

Table 2. The most frequent neuropsychiatric symptoms in AINS patients

AINS symptoms grouped	No of patients	%
Altered levels of consciousness	147	54 %
Confusion	146	53 %
Hallucinations	95	35 %
Involuntary movements	79	29 %
Impaired coordination	63	23 %
Agitation	50	18 %
Speech disorder	47	17 %
Incoherence	33	12 %
Apathy	32	12 %

We also found that the ratio between serum CMMG and ACV concentrations in AINS subjects was 3.31 ± 3.29. The corresponding value for patients without known neuropsychiatric adverse reactions in our TDM database was 0.68 ± 4.10 ($p<0.001$). This study confirms that altered level of consciousness, confusion, and hallucinations are the most frequent clinical characteristics of AINS. Renal failure is a major risk factor.

We found that more than 80 % of HSE patients presented with high fever, altered level of consciousness, focal neurological signs, and headache (Table 3). Altered level of consciousness was also the most frequent symptom in AINS patients, but to a lesser extent (54 %). Other frequent symptoms, such as visual and auditory hallucinations,

involuntary movements, impaired coordination, and agitation was present in AINS patients, but not in HSE patients. Importantly, the frequency of confusion/disorientation was not significantly different from patients with HSE.

Table 3. The most frequent signs and symptoms in patients with herpes encephalitis (HSE)

HSE symptoms grouped	%
Fever	99 %
Altered level of consciousness	95 %
Focal neurological signs	89 %
Headache	77 %
Autonomic-nervous system dysfunction	75 %
Confusion/Disorientation	72 %
Personality changes	69 %
Ataxia	67 %
Meningism	60 %
Seizures	58 %

3.10 PAPER V

3.10.1 Study design

Even if our earlier studies supported the primary hypothesis of a correlation between CMMG and AINS, it was considered particularly important to obtain detailed description of the pharmacokinetics (PK) of CMMG and its relation to renal function. Thus, we performed a PK study including patients with various degrees of renal function. Paper V describes a single-dose, open-label PK crossover study consisting of one i.v. part and one oral. Exposure (AUC), bioavailability, half-life, CL and several other PK parameters were estimated. Renal function was estimated using measured creatinine clearance, Cockroft-Gaults equation [125], MDRD [120], and CKD-Epi [126].

3.10.2 Preliminary results

Sixteen patients were included in the study: six healthy volunteers with an estimated GFR above 70 mL/min, six volunteers with chronic kidney disease (CKD) with a GFR between 60 to 20 mL/min, and four patients on HD. The mean CMMG levels in HD patients were increased more than 10-fold compared to healthy volunteers (Figure 7). Subjects with normal and even patients with impaired renal function had CMMG levels close to or below LOQ.

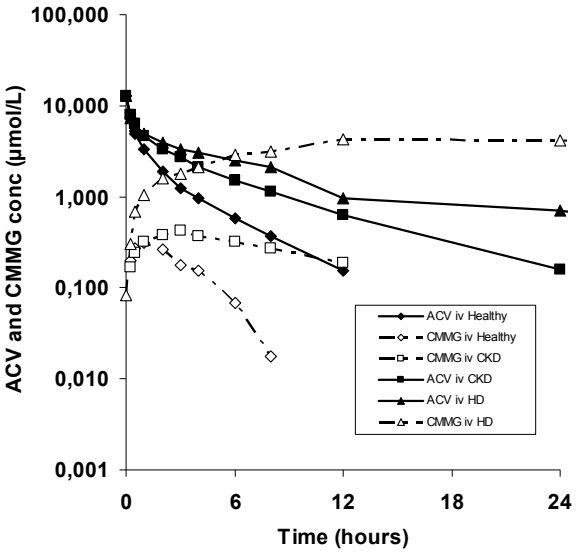

Figure 7. ACV and CMMG concentrations after a single intravenous administration of 100 mg ACV to six healthy subjects and 50 mg to six subjects with impaired renal function (CKD) and four HD-patients. The ACV metabolite CMMG accumulates in HD patients, while both healthy and CKD subjects show low and decreasing concentrations.

3.10.3 ACV and CMMG PK and renal function

ACV and CMMG PK depended heavily on renal function (Figure 8). Bioavailability was significantly higher in HD patients than in CKD patients (p= 0.021) despite the fact that they had received the same dose. A single HD decreased the ACV and CMMG serum concentrations by 56 and 73 %, respectively. We conclude that dose regimens aiming to minimize CMMG exposure can be developed based on these data. This will allow further examinations of the causality of CMMG in AINS and studies on how to avoid it.

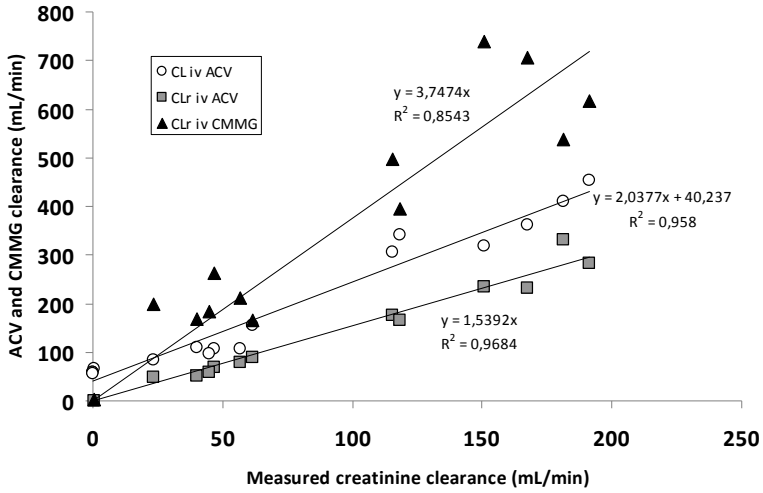

Figure 8. The relation between measured creatinine clearance on X-axis and total ACV-CL (open circles), renal ACV-CLr (squares) and renal CMMG-CLr (triangles) on Y-axis in study V.

4 DISCUSSION

The hypothesis presented in this thesis is that aciclovir-induced adverse reactions emanates from increasing concentrations of the ACV main metabolite CMMG. As with all ADRs, the diagnosis of AINS has been a differential diagnosis [127]. It has been based on the pattern of neuropsychiatric symptoms [81,91], negative blood and CSF tests for viral infection, improvement after discontinuation of ACV, or by HD [99,103,117,128,129]. However, the diagnosis has in some cases been confused with symptoms from the underlying disease (confounding by indication), resulting in increased doses of ACV instead of withdrawal [7,8,130]. This has lead to a demand for a test to diagnose AINS.

This thesis suggests that a simple, fast, and reliable diagnostic test for measurement of CMMG and ACV in serum, CSF, and urine, may provide valuable information to solve the longstanding problem of diagnosing AINS. The conclusion that CMMG is either important for the development, or a marker, of AINS is based on ADR case reports, pharmacoepidemiology studies, TDM data, and a pharmacokinetic study.

4.1 PAPER I

In the first paper (Paper I) we found that patients with AINS had significantly higher CMMG serum concentrations than did asymptomatic patients. A conventional ROC analysis showed that a CMMG serum cut-off value of roughly 11 µmol/L had a higher sensitivity and specificity to predict AINS than ACV exposure, ACV concentrations and renal function tests. However, a S-creatinine value above 251 µmol/L and Cl_{Cr} < 17 mL/min had a sensitivity of 94 % and 88 %, respectively, indicating that impaired renal function played an important role in the development of AINS. Despite the fact that several patients received doses adapted to renal function, the significantly higher ACV exposure in the AINS group, compared to the asymptomatic group, raised the question if these patients had divergent bioavailability. Asymptomatic patients had a significantly higher GFR compared to AINS patients, 29.6 ± 27.4 mL/min/1.73 m^2 and 19.5 ± 21.1 mL/min/1.73 m^2, respectively (p=0.04) (Figure 9).

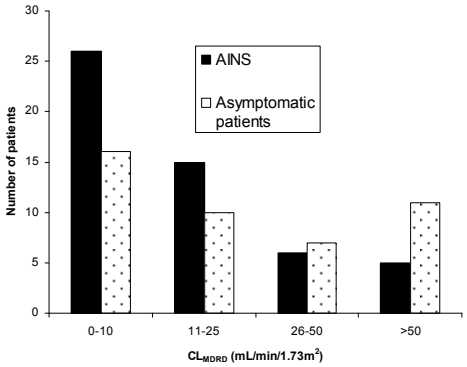

Figure 9. Renal function calculated by the MDRD formula in patients with AINS and asymptomatic patients in study I.

It thus appeared that higher oral ACV doses increased the risk of developing AINS. More surprising was the finding that neither the oral VACV, nor the i.v. ACV dose differed significantly between the two groups. Again, impaired renal function seemed to be an important factor predisposing for the development of AINS. The symptom profile of AINS did not differ from published cases, except for a few symptoms such as pronounced tiredness, somnolence and restlessness. This discrepancy probably reflected a difference in describing symptoms in the patient charts. This problem was even more pronounced in paper IV, where we compared symptoms described in our Swedish cases and in case reports.

4.1.1.1 Outcome

Thirty-nine out of the 49 AINS patients recovered. Six patients died in close connection to the adverse event, four of them while unconscious and on ACV therapy. The number of deaths was unexpectedly high and none of them had signs or symptoms of CNS infection. Furthermore, three of the deceased and two of the surviving patients had developed cerebral lesions.

4.1.1.2 AINS, hypertension and posterior reversible encephalopathy syndrome (PRES)

Concerning the development of cerebral lesions in AINS patients one can speculate if high concentrations of ACV, or its metabolites, might cause hypertension in vulnerable subjects. In study IV we found that 32 of 275 patients (12 %) had increased blood pressure or malignant hypertension in close relation to the ADR event. We have reported a case of an aciclovir-induced MRI-verified posterior leucoencephalopathy, developing in a PD-patient with shingles. The patient became agitated, developed visual hallucinations and drowsiness, and had a blood pressure of 180/104 mm Hg. The highest ACV and CMMG concentrations were 35 μmol/L and 149 μmol/L, respectively. MRI showed posterior white matter changes, predominantly in the occipital and parietal lobes [131].

Heye *et al* reported a case of vasculitic, hypoxic-ischemic leucoencephalopathy in a 67-year-old woman with HD on ACV [132]. In addition, Blohm et al reported a 12-year-old girl who developed focal secondary generalizing epileptic fits, following a 4 week prophylactic administration of ACV. Brain MRI demonstrated multiple gadolinium-enhancing areas, with impairment of the blood-brain barrier in cortical and subcortical regions. Clinical symptoms and neuroimaging pathology resolved completely within 9 days of acyclovir withdrawal [97].

AINS share some of the features of posterior reversible encephalopathy syndrome (PRES), i.e. confusion, seizures, and posterior transient changes on neuroimaging [131]. It has been suggested that PRES is related to the breakthrough of autoregulation in the brain and endothelial dysfunction [133]. It is important to stress the fact that increased blood pressure might be caused by the neuropsychiatric symptoms, e.g. anxiety and hallucinations, occurring in AINS. However, the increased blood pressure seen in AINS patients eventually returned to its previous level when the ACV and CMMG concentrations decreased.

4.2 PAPER II

To our knowledge Paper II was the first study to demonstrate the presence of CMMG in CSF. The result provided further support for the primary hypothesis that CMMG is involved in the development of neuropsychiatric side effects in ACV- or VACV-treated patients. Another finding was the linear correlation between CMMG serum and CSF concentrations.

Recently Smith and colleagues presented a study on PK of ACV and its metabolites after the administration of high-dose VACV in subjects with normal and impaired renal function [134]. Subjects with normal renal function had a maximum CMMG concentration (Cmax) in CSF of 0.095 ± 0.046 µmol/L (range 0.034 – 0.16). CKD patients had a higher Cmax: 0.37 ± 0.20 (0.17-0.58) µmol/L. On the whole both groups exhibited CMMG concentrations below the LOQ we used in our study (<0.5 µmol/L). The ACV CSF-to-plasma AUC ratio in healthy subjects and CKD patients were 0.27 and 0.28, respectively, while the corresponding values for CMMG CSF-to-plasma AUC ratio were 0.025 and 0.026, respectively. CSF concentrations of ACV, as well as CMMG and the second ACV metabolite 8-OH-ACV, were stable during the study. Similar results were reported by Lycke and colleagues, suggesting a slow transport in and out of CSF [115]. This slow transport has also been discussed in a review article by Löscher and Potschka [135].

Data from 13 AINS patients included in study IV showed a mean ACV CSF-serum ratio of 0.20 ± 0.17. In study II the ACV CSF-to-serum ratio in asymptomatic patients as well as in AINS patients were median 0.3 (range 0.2 to 1.46) and 0.46 (range 0.23 to 2.50), respectively. It can also be noted that describing the distribution of ACV and CMMG across the BBB as a constant ratio is apparently inaccurate, as the fluctuations in serum is much larger than the fluctuation in the CSF. This may explain much of the confusion in the published literature regarding the BBB distribution of ACV.

4.2.1.1 Drug transporters in the brain

The results in paper II cannot be discussed without a brief description of drug transporters in the brain. I will focus on the blood-brain barrier (BBB) as a protection barrier, preventing toxic substances to enter the brain. BBB forms a diffusion barrier with tight junctions between the endothelial cells. Lipophilic compounds with low molecular weight may enter the brain by passive diffusion, while other lipophilic compounds cannot, due to transporter protein activity. The barrier consists not only of tight junctions preventing substances to enter, but also of proteins that function as transporters. There are several transporters present in the BBB, but also in the blood-CSF barrier. They regulate the in- and out flux of substances from the blood to the brain tissue.

Among these transporters P-glycoprotein (Pgp), multidrug resistance proteins (MRPs) and anion and cation transporting polypeptides (OAT and OCT) are the most important and most studied. Pgp is mainly expressed at the apical side of the brain capillary endothelial cells, pumping out Pgp substrates back to the blood. Inhibition of Pgp may lead to increased concentrations of a toxic substance or drug in the brain [135].

It has been suggested that ACV is an inhibitor of OAT3. It was shown in an *in vitro*-study that the transport of indoxyl sulphate, a uremic toxin found in renal failure patients and a marker substance for neurotransporter metabolites, was inhibited by ACV. This indicates that ACV may increase the accumulation of these substances in the brain [136]. If this is applicable also to CMMG accumulation needs to be elucidated.

4.2.1.2 Renal function

Ten of the 12 asymptomatic patients in paper II have earlier been described concerning ACV concentrations, but without measurement of CMMG. Their estimated renal function was 90 ± 21 mL/min. Their mean (± SD) ACV and CMMG serum concentrations are shown in Figure 10.

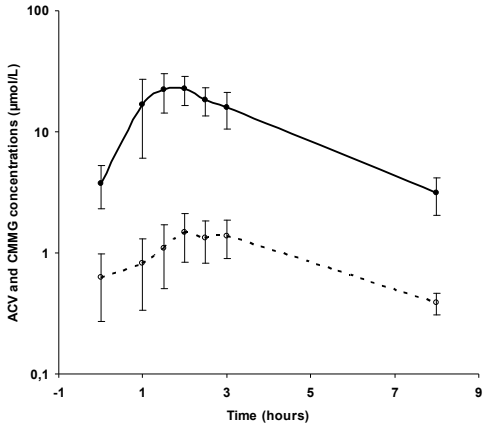

Figure 10. The figure shows ACV and CMMG concentrations in patients with normal renal function on VACV. The CMMG serum concentrations are low and in line with previous publications [38]. The parallel disappearance of ACV and CMMG suggests that the kinetics of CMMG in healthy subjects with normal renal function is formation and not elimination dependent. Observe the log scale on the Y-axis. ACV = straight line, CMMG= dotted line.

4.2.1.3 Conclusions from paper II

The results from paper II and the discussion in the paragraphs above can be summarized as follow:

- CMMG could be found in CSF only in patients with AINS.
- All but one patient with AINS had impaired renal function.
- CMMG serum levels were low in asymptomatic subjects, while the mean values were 90 times higher in AINS patients.
- Measurement of CMMG in CSF might be the most accurate method to diagnose AINS.
- Inhibition of transporter proteins in the brain tissue may be involved in the development of AINS, but this needs to be elucidated.

4.3 PAPER III

Paper III describes two patients with Cotard's syndrome. After the initiation of VACV, in one case followed by i.v. ACV, both patients experienced a strong belief that they were dead. We could show a close relation between high CMMG concentrations in serum and the experienced symptoms. The implication that an ADR may cause the syndrome has not been shown before.

4.3.1 The syndrome

Cotard's syndrome was first described by the French neurologist Jules Cotard in 1880. He presented a female patient with "délire des negations" [137,138]. The syndrome "comprises any one of a series of delusions ranging from the fixed and unshakable belief that one has lost organs, blood, or body parts to believing that one has lost one's soul or is dead" [139]. Cotard's can persist for a long period, even for years. However, in our female patient it disappeared already after a single dialysis, suggesting that there is a concentration dependent effect on the brain from ACV or CMMG. Cotard's syndrome has been observed in schizophrenia, melancholic depression and other psychiatric diseases, but also in other somatic conditions such as Parkinson's disease and in patients with cerebral lesions. It is a rare condition and less than one case per year has been published since 1880. Interestingly, we found in study IV that depression and suicidal ideation were present in 5 % of the 275 investigated patients. Only recently Asahi and colleagues reported a 73-year-old male HD patient who developed confusion, hallucinations and a fear of "going to die" after VACV was initiated in a dose of 3000 mg/day [140]. Apparently, ACV treatment in ESRD patients may induce symptoms of depression, which disappear within a few hours on dialysis.

4.3.2 Might cerebral lesions be the cause?

Cotard's syndrome has been linked to damage of the non-dominant temporo-parietal cerebral cortex. Hypoperfusion in the temporal, parietal and frontal lobes have also been reported, which almost disappeared in one case after successful ECT (electro-convulsive therapy) treatment [141].

4.4 PAPER IV

Paper IV describes aciclovir-induced adverse symptoms in 275 patients and also presents a comparison between AINS and symptoms reported for HSE. One of the databases, SWEDIS, is not as comprehensive in its structure as published case reports. The most important symptoms were however noted in these ADR reports, and information could also be extracted from the text in the report.

Two important findings in the study were that the majority of AINS patients were treated with oral ACV or VACV and that shingles was the most common indication. Women received the same doses as men. The annual incidence to develop shingles increases with age, as shown in paragraph 1.4.1. Elderly women with age-related kidney function decline may be more vulnerable to develop AINS. Renal function in elderly patients should therefore always be checked before and during treatment with ACV or VACV. We have recently shown that many elderly patients developing ARDs had low renal function [142].

Our study also showed that HSE patients usually presented with distinct symptoms such as high fever, altered level of consciousness, focal neurological signs, and headache. Other symptoms more frequently observed in HSE patients were seizures, vomiting and hemiparesis. However, as many as 50 % of HSE and AINS patients had confusion and altered level of consciousness, making it difficult to distinguish AINS from HSE in these cases.

4.4.1 Other symptoms

Forty-seven patients (17 %) developed gastrointestinal symptoms and 44 patients developed hypoxia, or lung symptoms, generally requiring mechanical ventilation. The mechanism behind this is still unknown, but similar cases have been reported [143].

4.4.2 Investigations

The results from investigations performed in AINS patients, such as CT, MRI, and lumbar puncture, are in most cases normal and help to facilitate the diagnostic procedure. EEG in HSE may show lateralisation and periodic spike and slow-wave activity emanating from the infected hemisphere. However, EEG has a sensitivity of approximately 84 %, but specificity is not higher than 33 % [12]. EEG in AINS patients is in general described as metabolic encephalopathy with diffuse slowing [81].

4.4.3 AINS and delirium

We found the grading of neuropsychiatric symptoms to be difficult. Many aciclovir-induced adverse reactions corresponded to symptoms present in delirium, such as confusion, psychosis, disorientation, and frightening visual hallucinations. Several formal instruments have been developed to diagnose delirium. These are aiming to help the clinicians to support the diagnosis of a condition which is complicated to assess. In paper IV we used four different assessment scales, but none of them could entirely cover all the different symptoms present in AINS.

4.4.4 Conclusion paper IV

We conclude that it is difficult to distinguish between CNS symptoms in patients with HSE and patients with AINS. This view was also put forward as it was found that the clinical findings of HSE are non-specific, and that the symptom profile itself cannot be the only tool to support the diagnosis [12]. The AINS profile presented in paper IV is a valuable tool in the diagnosis of AINS, but may alone be insufficient when distinguishing from herpes or varicella encephalitis. Therefore TDM of ACV, and in particular, its main metabolite CMMG, is recommended.

4.5 PAPER V

In paper V the PK of ACV and CMMG were studied in patients with various degrees of renal function, aiming to provide PK parameter estimates for developing ACV dose recommendations. Our goal was to find an ACV concentration above the inhibitory dose (ID_{50}) for HSV and VZV, but without increased concentrations of CMMG and risk for AINS. Low doses were used to avoid the development of AINS in the participants. This resulted in low CMMG concentrations in subjects with normal renal function and patients with moderately impaired renal function. However, ACV concentrations were above, or in line with, IC_{50} for HSV and many susceptible VZV strains.

For some individuals the parameter estimates are uncertain. Nevertheless, the relation between renal function and the clearance of both ACV and CMMG could in some cases be established and was linear. The results obtained can be utilized to develop dose regimens aimed to maximize the ACV exposure compared to CMMG exposure.

4.6 OTHER ASPECTS OF AINS

4.6.1 How to explain the lack of relation between high ACV concentrations and AINS?

The RIA methods had high sensitivity and specificity, and could separate ACV from CMMG. As mentioned earlier, there had only been one instance where CMMG had been found in the serum of a patient [64]. The HPLC assay developed by Svensson *et al* did also have high sensitivity and specificity, and furthermore, it could also separate CMMG from ACV. This method was unique and other laboratories did not have this facility.

A French AINS case can illustrate the problem with a HPLC method which cannot separate ACV from CMMG. A 64-year-old woman on HD developed altered level of consciousness four days after the initiation of i.v. ACV, 5mg/kg qid. ACV concentrations were initially analyzed by a HPLC method. The first sample was collected two days after discontinuation of ACV, the remaining two after two separate HD sessions. The analysis of ACV showed 80, 98, and 124 μmol/L, respectively. Surprisingly the ACV concentrations increased despite discontinuation of therapy and HD. When the same samples were analyzed by the more sensitive RIA method, they showed 25, 2.8 and 0.53 μmol/L, respectively [112], similar to the decrease reported earlier after HD [117]. A reasonable hypothesis is that the HPLC method did not separate between ACV and CMMG, and that the increasing ACV levels represented CMMG concentrations.

4.6.2 Treatment of AINS

ACV should be discontinued in a patient developing AINS and dehydrated patients should receive fluid. Forced diuresis has been tried in patients with AINS and ARF, but the rational for this measure is unclear and has not been studied in AINS patients. Cases with breathing difficulties, confusion, stupor, and coma have been reported to benefit from a 3-4 hrs HD, which may be repeated if the patient does not recover after the first HD session [103].

4.6.2.1 Low dose ACV in ESRD patients.

Doses lower than recommended in the PDR has been tried in patients on HD to avoid AINS. Almond *et al* performed a pharmacokinetic study in seven HD-patients. Computer modelling showed that sufficient ACV concentrations could be achieved with a loading dose of 400 mg, and a maintenance dose of 200 mg twice daily. The model suggests that a further loading dose of 400 mg should be administered after each dialysis session [144]. We tried the dose recommendations presented in the study and measured ACV and CMMG concentrations in two patients, one on HD and the other on PD. Figure 11a shows that ACV concentrations were within the range suggested to be effective against HSV and VZV. CMMG concentrations were low and not in the range seen in patients with AINS. Both ACV and CMMG concentrations were increasing in the PD patient, suggesting that PD patients may pose an increased risk for developing AINS. However, this has not been evaluated in prospective studies (Figure 11b)

11a 11b

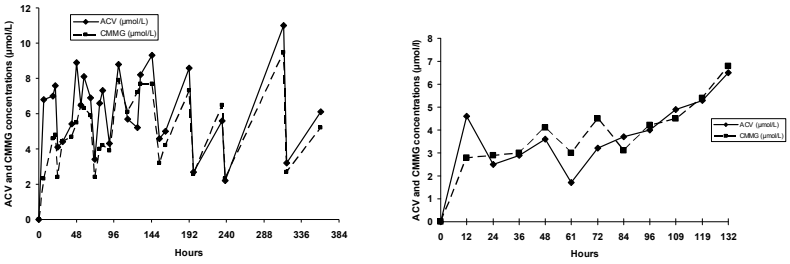

Figure 11. a) ACV and CMMG concentrations in a hemodialysis patient given oral ACV in a dose of 200 mg x 2. b) ACV and CMMG concentrations in a patient on peritoneal dialysis administered oral ACV in a dose of 200 mg x 2. ACV and CMMG show increasing concentrations, beginning four days after the initiation of the therap. This may increase the risk for developing AINS. ACV clearance during PD is approximately 3 mL/min [145]. Hence, regular PD cannot be used to decrease increased concentrations of ACV and CMMG.

4.6.3 Are there any other possible causes to the development of AINS?

It is evident that there may be several other factors that may contribute to the development of AINS. We have focused on the relation between AINS and CMMG. We cannot rule out that the second ACV metabolite 8-OH-ACV may be responsible for the development of AINS. It is also possible that the transport of other substances in the brain causing AINS might be inhibited by ACV or any of its metabolites. Direct studies of the effects of CMMG *per se* are obviously needed to further illuminate the mechanisms involved.

Importantly, the present data does not provide direct evidence that CMMG is the cause of AINS. There are some publications arguing in favour of the CMMG hypothesis [114,131,146-148], but there are also alternative hypotheses. In one study it was found that there were increased concentrations of CMMG in hemodialysis patients who did not exhibit neuropsychiatric reactions [134]. There is no obvious explanation to this discrepancy, and apparently further studies are needed to clarify the role of CMMG in AINS.

5 FUTURE PERSPECTIVES AND GENERAL CONCLUSIONS

5.1 FUTURE PERSPECTIVES

A large number of aspects of AINS suggest future studies. Some of these are included below:

- To measure neurotransmitter and/or its metabolites in AINS patients.
- To study the impact of drug transporters in the BBB and blood-CSF barrier and the development of AINS.
- To study the metabolism of ACV in different ethnic groups to better predict the development of AINS.
- To develop a new assessment scale for AINS events.
- To study possible toxicity of CMMG.
- To study the presence and impact of the ACV aldehyde.
- To study the presence and impact of the 8-OH-ACV metabolite.

5.2 CONCLUSIONS

From the results obtained in this thesis it is concluded that:

- The ACV main metabolite 9-carboxymethoxymethylguanine (CMMG) is consistently increased in patients with AINS.
- CMMG has a higher sensitivity and specificity as a predictor of AINS than ACV concentrations and estimates of renal function.
- CMMG, as well as ACV, enters the brain and can be detected in CSF.
- Impaired renal function appears to be the main risk factor for development of AINS.
- AINS may present as a delusion of being dead.
- AINS are most common in patients with herpes zoster and after oral treatment.
- The most frequent symptoms seen in AINS patients are altered level of consciousness, confusion, hallucinations and involuntary movements, while patients with HSE have significantly higher frequency of high fever, altered level of consciousness, focal neurological signs, and headache.
- AINS may be difficult to distinguish from herpes encephalitis, and probably also other viral CNS infections, if the assessment is based solely on the symptom profiles.
- Dose regimens aimed to minimize CMMG exposure may be developed based on the pharmacokinetic results.
- The results of this thesis support the hypothesis that CMMG is a useful predictor of AINS and it may be mechanistically involved. Determination of CMMG concentrations may be a useful tool to distinguish between AINS and symptoms from viral CNS infections.

Finally, this thesis is an example of how serendipity (the ability to make a discovery by a lucky chance) and curiosity can create a new field of research.

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7 REFERENCES

[1]. De Clercq E. Antiviral drugs in current clinical use. *J Clin Virol* 2004;30(2):115-33.

[2]. Tilson HH. Monitoring the safety of antivirals. The example of the acyclovir experience. *Am J Med* 1988;85(2A):116-22.

[3]. Brigden D, Rosling AE, Woods NC. Renal function after acyclovir intravenous injection. *Am J Med* 1982;73(1A):182-5.

[4]. Sawyer MH, Webb DE, Balow JE, Straus SE. Acyclovir-induced renal failure. Clinical course and histology. *Am J Med* 1988;84(6):1067-71.

[5]. Feldman S, Rodman J, Gregory B. Excessive serum concentrations of acyclovir and neurotoxicity. *J Infect Dis* 1988;157:385-8.

[6]. Bataille P, Devos P, Noel JL, Dautrevaux C, Lokiec F. Psychiatric side-effects with acyclovir. *Lancet* 1985;II:724.

[7]. Collins A. Neuropsychiatric toxicity in a patient undergoing hemodialysis and receiving treatment with oral acyclovir. *Clin Infect Dis* 1996;22(1):187-8.

[8]. Guitton C, Maury E, Mohand D et al. Acute reversible neurological and renal failure following acyclovir treatment. *Intensive Care Med* 1999;25(6):639-40.

[9]. Fiddian AP, Brigden D, Yeo JM, Hickmott EA. Acyclovir: an update of the clinical applications of this antiherpes agent. *Antiviral Res* 1984;4(3):99-117.

[10]. Steiner I, Kennedy PG, Pachner AR. The neurotropic herpes viruses: herpes simplex and varicella-zoster. *Lancet Neurol* 2007;6(11):1015-28.

[11]. Levitz RE. Herpes simplex encephalitis: a review. *Heart Lung* 1998;27(3):209-12.

[12]. Whitley RJ. Herpes simplex encephalitis: adolescents and adults. *Antiviral Res* 2006;71(2-3):141-8.

[13]. Smith MG, Lennette EH, Reames HR. Isolation of the virus of herpes simplex and the demonstration of intranuclear inclusions in a case of acute encephalitis. *Am J Pathol* 1941;17(1):55-68.

[14]. Kimberlin DW. Management of HSV encephalitis in adults and neonates: diagnosis, prognosis and treatment. *Herpes* 2007;14(1):11-6.

[15]. Chaudhuri A, Kennedy PG. Diagnosis and treatment of viral encephalitis. *Postgrad Med J* 2002;78(924):575-83.

[16]. Hjalmarsson A, Blomqvist P, Sköldenberg B. Herpes simplex encephalitis in Sweden, 1990-2001: incidence, morbidity, and mortality. *Clin Infect Dis* 2007;45(7):875-80.

[17]. Aurelius E, Johansson B, Sköldenberg B, Forsgren M. Encephalitis in immunocompetent patients due to herpes simplex virus type 1 or 2 as determined by type-specific polymerase chain reaction and antibody assays of cerebrospinal fluid. *J Med Virol* 1993;39(3):179-86.

[18]. Whitley RJ, Soong SJ, Dolin R, Galasso GJ, Ch'ien LT, Alford CA. Adenine arabinoside therapy of biopsy-proved herpes simplex encephalitis. *J Med Assoc State Ala* 1977;47(3):37-42 66.

[19]. Sköldenberg B, Forsgren M, Alestig K et al. Acyclovir versus vidarabine in herpes simplex encephalitis. Randomised multicentre study in consecutive Swedish patients. *Lancet* 1984;2(8405):707-11.
[20]. Kennedy PG. A retrospective analysis of forty-six cases of herpes simplex encephalitis seen in Glasgow between 1962 and 1985. *Q J Med* 1988;68(255):533-40.
[21]. McGrath N, Anderson NE, Croxson MC, Powell KF. Herpes simplex encephalitis treated with acyclovir: diagnosis and long term outcome. *J Neurol Neurosurg Psychiatry* 1997;63(3):321-6.
[22]. Tunkel AR, Glaser CA, Bloch KC et al. The management of encephalitis: clinical practice guidelines by the Infectious Diseases Society of America. *Clin Infect Dis* 2008;47(3):303-27.
[23]. Steiner I, Budka H, Chaudhuri A et al. *Eur J Neurol* 2010;.
[24]. Welsby PD. Chickenpox, chickenpox vaccination, and shingles. *Postgrad Med J* 2006;82(967):351-2.
[25]. Chapman RS, Cross KW, Fleming DM. The incidence of shingles and its implications for vaccination policy. *Vaccine* 2003;21(19-20):2541-7.
[26]. Johnson RW. Herpes zoster and postherpetic neuralgia. *Expert Rev Vaccines* 2010;9(3 SUPPL):21-6.
[27]. Morse GD, Shelton MJ, O'Donnell AM. Comparative pharmacokinetics of antiviral nucleoside analogues. *Clin Pharmacokinet* 1993;24(2):101-23.
[28]. Hoglund M, Ljungman P, Weller S. Comparable aciclovir exposures produced by oral valaciclovir and intravenous aciclovir in immunocompromised cancer patients. *J Antimicrob Chemother* 2001;47(6):855-61.
[29]. King DH. History, pharmacokinetics, and pharmacology of acyclovir. *J Am Acad Dermatol* 1988;18(1 PT 2):176-9.
[30]. Schaeffer HJ, Beauchamp L, de Miranda P, Elion GB, Bauer DJ, Collins P. 9-(2-hydroxyethoxymethyl) guanine activity against viruses of the herpes group. *Nature* 1978;272(5654):583-5.
[31]. Elion GB, Furman PA, Fyfe JA, de Miranda P, Beauchamp L, Schaeffer HJ. Selectivity of action of an antiherpetic agent, 9-(2-hydroxyethoxymethyl) guanine. *Proc Natl Acad Sci U S A* 1977;74(12):5716-20.
[32]. Elion GB. Mechanism of action and selectivity of acyclovir. *Am J Med* 1983;73(1A):7-13.
[33]. Furman PA, de Miranda P, St Clair MH, Elion GB. Metabolism of acyclovir in virus-infected and uninfected cells. *Antimicrob Agents Chemother* 1981;20(4):518-24.
[34]. Smee DF, Boehme R, Chernow M, Binko BP, Matthews TR. Intracellular metabolism and enzymatic phosphorylation of 9-(1,3-dihydroxy-2-propoxymethyl)guanine and acyclovir in herpes simplex virus-infected and uninfected cells. *Biochem Pharmacol* 1985;34(7):1049-56.
[35]. de Miranda P, Blum MR. Pharmacokinetics of acyclovir after intravenous and oral administration. *J Antimicrob Chemother* 1983;12 Suppl B:29-37.

[36]. O'Brien JJ, Campoli-Richards DM. Acyclovir. An updated review of its antiviral activity, pharmacokinetic properties and therapeutic efficacy. *Drugs* 1989;37(3):233-309.

[37]. de Miranda P, Good SS, Laskin LO, Krasny HH, Connor DJ, Lietman SP. Disposition of intravenous radioactive acyclovir. *Clin Pharmacol Ther* 1981;30:662-72.

[38]. Soul-Lawton J, Seaber E, On N, Wootton R, Rolan P, Posner J. Absolute bioavailability and metabolic disposition of valaciclovir, the L-valyl ester of acyclovir, following oral administrations to humans. *Antimicrob Agents Chemother* 1995;39:2759-64.

[39]. de Miranda P, Good S. Biotransformation of acyclovir to 9-carboxymethoxymethylguanine (abstract). *Fed Proc* 1982;41:1733.

[40]. de Miranda P, Good SS. Speciec differences in the metabolism and disposition of antviral nucleoside analogues: 1. Acyclovir. *Antivir Chem Chemother* 1992;3(1):1-8.

[41]. Ramchandani VA, Bosron WF, Li TK. Research advances in ethanol metabolism. *Pathol Biol (Paris)* 2001;49(9):676-82.

[42]. Laskin OL, Longstreth JA, Whelton A et al. Acyclovir kinetics in end-stage renal disease. *Clin Pharmacol Ther* 1982;31(5):594-601.

[43]. Zeng L, Nath CE, Blair EY et al. Population pharmacokinetics of acyclovir in children and young people with malignancy after administration of intravenous acyclovir or oral valacyclovir. *Antimicrob Agents Chemother* 2009;53(7):2918-27.

[44]. de Miranda P, Whitley RJ, Blum MR et al. Ayclovir kinetics after intravenous infusion. *Clin Pharmacol Ther* 1979;26:718-28.

[45]. Wade JC, Hintz M, McGuffin R, Springmeyer SC, Connor JD, Meyers JD. Treatment of cytomegalovirus pneumonia with high-dose acyclovir. *Am J Med* 1982;73(1A):249-56.

[46]. Laskin OL, Longstreth JA, Whelton A et al. Effect of renal failure on the pharmacokinetics of acyclovir. *Am J Med* 1982;73(1A):197-201.

[47]. Peterslund N, Black F, Tauris P. Impaired renal function after bolus injections of acyclovir. *Lancet* 1983;29:243-4.

[48]. Wade JC, McGuffin RW, Springmeyer SC, Newton B, Singer JW, Meyers JD. Treatment of cytomegaloviral pneumonia with high-dose acyclovir and human leukocyte interferon. *J Infect Dis* 1983;148(3):557-62.

[49]. Cohen S, Minkove J, Zebley J3, Mulholland J. *Ann Intern Med* 1984;100.

[50]. Kumor K, Conklin R, Woo J, Katz L, Strocchia C. Renal function studies during intravenous acyclovir treatment of immune suppressed patients including renal transplantation. *Am J Nephrol* 1988;8(1):35-9.

[51]. Yarlagadda SG, Perazella MA. Drug-induced crystal nephropathy: an update. *Expert Opin Drug Saf* 2008;7(2):147-58.

[52]. Ahmad T, Simmonds M, McIver AG, McGraw ME. Reversible renal failure in renal transplant patients receiving oral acyclovir prophylaxis. *Pediatr Nephrol* 1994;8(4):489-91.

[53]. Perazella M. Crystal-induced acute renal failure. *Am J Med* 1999;106:459-65.

[54]. Peyriere H, Branger B, Bengler C et al. Toxicite neurologique induite par le Zelitrex (valaciclovir) chez trois patients insuffisants renaux. Surdosage lie a l'amelioration de la biodisponibilite du produit? *Rev Med Interne* 2000;28(3):297-303.

[55]. Spiegal D, Lau K. Acute renal failure and coma secondary to acyclovir therapy. *JAMA* 1986;255:1882-3.

[56]. Sugimoto T, Yasuda M, Sakaguchi M et al. Oliguric acute renal failure following oral valacyclovir therapy. *QJM* 2008;101(2):164-6.

[57]. Gunness P, Aleksa K, Kosuge K, Ito S, Koren G. Comparison of the novel HK-2 human renal proximal tubular cell line with the standard LLC-PK1 cell line in studying drug-induced nephrotoxicity. *Can J Physiol Pharmacol* 2010;88(4):448-55.

[58]. TDM. 2010, http://medical-dictionary.thefreedictionary.com. Accessed 2010-07-19.

[59]. Gilden DH, Kleinschmidt-DeMasters BK, LaGuardia JJ, Mahalingam R, Cohrs RJ. Neurologic complications of the reactivation of varicella-zoster virus. *N Engl J Med* 2000;342(9):635-45.

[60]. Quinn RP, de Miranda P, Gerald L, Good SS. A sensitive radioimmunoassay for the antiviral agent BW248U [9-(2-hydroxyethoxymethyl)guanine]. *Anal Biochem* 1979;98(2):319-28.

[61]. Skubitz KM, Quinn RP, Lietman PS. Rapid acyclovir radioimmunoassay, using charcoal adsorption. *Antimicrob Agents Chemother* 1982;21(2):352-4.

[62]. Tadepalli SM, Quinn RP, Averett DR. A competitive enzyme-linked immunosorbent assay to quantitate acyclovir and BW B759U in human plasma and urine. *Antimicrob Agents Chemother* 1986;29(1):93-8.

[63]. Mascher H, Kikuta C, Metz R, Vergin H. New, high-sensitivity high-performance liquid chromatographic method for the determination of acyclovir in human plasma, using fluorometric detection. *J Chromatogr* 1992;583(1):122-7.

[64]. Krasny HC, Petty BG. Metabolism of desciclovir, a prodrug of acyclovir, in humans after mutiple oral dosing. *J Clin Pharmacol* 1987;27:74-7.

[65]. Barkholt L, Lewensohn-Fuchs I, Ericzon BG, Tyden G, Andersson J. High-dose acyclovir prophylaxis reduces cytomegalovirus disease in liver transplant patients. *Transpl Infect Dis* 1999;1(2):89-97.

[66]. Svensson JO, Barkholt L, Säwe J. Determination of acyclovir and its metabolite 9-carboxymethoxymethylguanine in serum and urine using solid-phase extraction and high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 1997;690(1-2):363-6.

[67]. Saral R, Burns W, Laskin O, Santos G, Lietman P. Acyclovir prophylaxis of herpes-simplex-virus infections: a randomized double-blind controlled trial in bone-marrow transplant recipients. *N Engl J Med* 1981;305:63-7.

[68]. Bach MC. Possible drug interactions during therapy with azidothymidine and acyclovir for AIDS. *N Engl J Med* 1982;316(9):547.

[69]. Auwerx J, Knockaert D, Hofkens P. Acyclovir and neurologic manifestations. *Ann Intern Med* 1983;99:6:882-3.
[70]. Wade J, Meyers J. Neurologic symptoms associated with parenteral acyclovir treatment after marrow transplantation. *Ann Intern Med* 1983;98:6:921-5.
[71]. Vartian C, Shlaes D. Intravenous acyclovir and neurologic effects. *Ann Intern Med* 1983;99(4):568.
[72]. Tomson C, Goodship T, Rodger R. Psychiatric side-effects of acyclovir in patients with chronic renal failure. *Lancet* 1985;17:385-6.
[73]. Krigel R. Reversible neurotoxicity due to oral acyclovir in a patient with chronic lymphocytic leukemia. *J Infect Dis* 1986;154:189.
[74]. Rubin J. Overdose with acyclovir in a CAPD patient. *Peritoneal Dial Bull* 1987;7(1):42-3.
[75]. Burguet A, Menget A, Fromentin C et al. Effets neurologiques de l'aciclovir apres greffe de moelle allogenique. *Arch Fr Pediatr* 1988;45(5):343-5.
[76]. Swan S, Bennett W. Oral acyclovir and neurotoxicity. *Ann Intern Med* 1989;111(2):2.
[77]. Fischer A. Toxicite renale et neurologique de l'acyclovir. A propos d'un cas. *Schweiz Med Wochenschr* 1990;120(33):1200-3.
[78]. Eck P, Silver S, Clark E. Acute renal failure and coma after a high dose of oral acyclovir. *N Engl J Med* 1991;325/16:1178-9.
[79]. Papadakis M, Fee P, Wilhelm T, Davenport A, Connolly J. Facial weakness in a haemodialysis patient. *Lancet* 2007;369(9562):714.
[80]. Martinez-Diaz GJ, Hsia R. *J Emerg Med* 2009;.
[81]. Rashiq S, Briewa L, Mooney M, Giancarlo T, Khatib R, Wilson F. Distinguishing acyclovir neurotoxicity from encephalomyelitis. *J Intern Med* 1993;234:507-11.
[82]. Weller S, Blum MR, Doucette M et al. Pharmacokinetics of the acyclovir pro-drug valaciclovir after escalating single- and multiple-dose administration to normal volunteers. *Clin Pharmacol Ther* 1993;54(6):595-605.
[83]. Linssen-Schuurmans C, van Kan E, Feith G, Uges D. Neurotoxicity caused by valacyclovir in a patient on hemodialysis. *Ther Drug Monit* 1998;30(4):385-6.
[84]. Izzedine H, Mercadal L, Aymard G et al. Neurotoxicity of valacyclovir in peritoneal dialysis: a pharmacokinetic study. *Am J Nephrol* 2001;22(3):162-4.
[85]. Maru MC, Fialkow RZ, Haria DM. Choreiform movements in dialysis patient taking valacyclovir and famciclovir. *South Med J* 2001;94(6):655.
[86]. Saurina A, Ramirez de Arellano M, de las Cuevas X. Neurotoxicidad post-primera dosis de valaciclovir via oral en una paciente en hemodialisis. *Nefrologia* 2001;23(9):83-4.
[87]. Okada T, Nakao T, Matsumoto H et al. Valacyclovir neurotoxicity in a patient with end-stage renal disease treated with continuous ambulatory peritoneal dialysis. *Clin Nephrol* 2002;91(1):168-70.

[88]. Olin JL, Gugliotta JL. Possible valacyclovir-related neurotoxicity and aseptic meningitis. *Ann Pharmacother* 2003;18(12):1814-7.
[89]. Mutsumi I, Takashi H, Shigeru S, Hiroshi M, Masanobu U. Neurotoxicity of valacyclovir in a patient on hemodialysis. *Neurological Medicine* 2003;58(3):327-9.
[90]. Catalano C, Conforto L. [Valaciclovir-related neuro- and nephro-toxicity in two geriatric patients] Abstract. *G Ital Nefrol* 2005;22 Suppl 31:S132-4.
[91]. Das V, Peraldi MN, Legendre C. Adverse neuropsychiatric effects of cytomegalovirus prophylaxis with valaciclovir in renal transplant recipients. *Nephrol Dial Transplant* 2006;21(5):1395-401.
[92]. Australian A. *Australian adverse drug reactions bulletin* 2000;19(2).
[93]. Bean B, Aeppli D, Balfour HH. Acyclovir in shingles. *J Antimicrob Chemother* 1983;12 Suppl B:123-7.
[94]. Haefeli WE, Schoenenberger RA, Weiss P, Ritz RF. Acyclovir-induced neurotoxicity: concentration-side effect relationship in acyclovir overdose. *Am J Med* 1993;305(1):212-5.
[95]. Gill M, Burgess E. Neurotoxicity of acyclovir in end stage renal disease. *J Antimicrob Chemother* 1990;25:300-1.
[96]. Abad S, Deray G, Lokiec F, Hamani A, Petitclerc T, Jacobs C. Coma induced by intravenous acyclovir in a hemodialyzed patient (letter)]. *Presse Med* 1997;22:1050.
[97]. Blohm ME, Nurnberger W, Aulich A, Engelbrecht V, Burdach S. Reversible brain MRI changes in acyclovir neurotoxicity. *Bone Marrow Transplant* 1997;197(4):1049-51.
[98]. Bradley J, Forero N, Hoen P, Escobar B, Balakuntalam S, Anzueto A. Progressive somnolens leading to coma in a 68-year-old-man. *Chest* 1997;112(2):538-40.
[99]. Chiang C, Fanf C, Hsu W, Chu T, Tsai T. Hemodialysis reverses acyclovir-induced nephrotoxicity and neurotoxicity. *Dial Transplant* 2003;32(10):624-7.
[100]. Da Conceicao M, Genco G, Favier JC, Verrot D, Pitti R. Toxicite cerebrale et renale de l'aciclovir chez un patient traite pour meningo-encephalite. *Ann Fr Anesth Reanim* 1999;27 Pt 2:996-9.
[101]. Delerme S, De Jonghe B, Proost O, Outin H. Acyclovir-induced coma in a young patient without preexisting renal impairment. *Intensive Care Med* 2002;28(5):661-2.
[102]. Fukunishi I, Inada T, Horie Y. Manic symptoms caused by acyclovir in a hemodialysis patient. *Nephron* 1994;67(4):494.
[103]. Hsu CC, Lai TI, Lien WC, Chen WJ, Fang CC. Emergent hemodialysis for acyclovir toxicity. *Am J Emerg Med* 2005;23(7):899-900.
[104]. Loughrey J, Coleman P, Donohue J, Marsh B. Coma secondary to aciclovir neurotoxicity. *Ir J Med Sci* 2001;170(1):69-70.
[105]. Rivkin AM. Valacyclovir-induced seizures in end-stage renal disease. *Ann Pharmacother* 2003;37(12):1913.
[106]. Strong D, Hébert D. Acute acyclovir neurotoxicity in a hemodialyzed child. *Pediatr Nephrol* 1997;11(6):741-3.

[107]. Guilhem I, Charasse C, Gary J et al. Neurotoxicite de l'aciclovir chez le dialyse peritoneal a propos d'un cas. *Nephrologie* 1990;120(33):241-3.
[108]. de Knegt R, van der Pijl H, van Es L. Acyclovir-associated encephalopathy, lack of relationship between acyclovir levels and symptoms. *Nephrol Dial Transplant* 1995;10:1775-7.
[109]. Beales P, Almond MK, Kwan JT. Acyclovir neurotoxicity following oral therapy: Prevention and treatment in patients on haemodialysis. *Nephron* 1994;66:362-3.
[110]. Chevret L, Debray D, Poulain C, Durand P, Devictor D. Neurological toxicity of acyclovir: report of a case in a six-month-old liver transplant recipient. *Pediatr Transplant* 2006;10(5):632-4.
[111]. Davenport A, Goel S, Mackenzie JC. Neurotoxicity of acyclovir in patients with end-stage renal failure treated with continuous ambulatory peritoneal dialysis. *Am J Kidney Dis* 1992;62(3):647-9.
[112]. Leikin J, Shicker L, Orlowski J, Blair A, McAllister K. Hemodialysis removal of acyclovir. *Vet Hum Toxicol* 1995;37(3):233-4.
[113]. MacDiarmaid-Gordon A, O' Connor M, Beaman M, Ackrill P. Neurotoxicity associated with oral acyclovir in patients undergoing dialysis. *Nephron* 1992;62:280-3.
[114]. Yang HH, Hsiao YP, Shih HC, Yang JH. Acyclovir-induced neuropsychosis successfully recovered after immediate hemodialysis in an end-stage renal disease patient. *Int J Dermatol* 2007;46(8):883-4.
[115]. Lycke J, Malmeström C, Ståhle L. Acyclovir levels in serum and cerebrospinal fluid after oral administration of valacyclovir. *Antimicrob Agents Chemother* 2003;47(8):2438-41.
[116]. SWEDIS. *http://swewebmpase/swedisii/*.
[117]. Helldén A, Odar-Cederlöf I, Diener P et al. High serum concentrations of the acyclovir main metabolite 9-carboxymethoxymethylguanine in renal failure patients with acyclovir-related neuropsychiatric side effects: an observational study. *Nephrol Dial Transplant* 2003;18(6):1135-41.
[118]. Helldén A, Lycke J, Vander T, Svensson JO, Odar-Cederlöf I, Ståhle L. The aciclovir metabolite CMMG is detectable in the CSF of subjects with neuropsychiatric symptoms during aciclovir and valaciclovir treatment. *J Antimicrob Chemother* 2006;57(5):945-9.
[119]. Helldén A, Odar-Cederlöf I, Larsson K, Fehrman-Ekholm I, Lindén T. Death delusion. *BMJ* 2007;335(7633):1305.
[120]. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med* 1999;130(6):461-70.
[121]. Schwartz GJ, Haycock GB, Edelmann CM, Spitzer A. A simple estimate of glomerular filtration rate in children derived from body length and plasma creatinine. *Pediatrics* 1976;58(2):259-63.
[122]. Griner PF, Mayewski RJ, Mushlin AI, Greenland P. Selection and interpretation of diagnostic tests and procedures. Principles and applications. *Ann Intern Med* 1981;94(4 PT 2):557-92.

[123]. Vander T, Medvedovsky M, Herishanu Y. Encephalopathy induced by oral acyclovir in a patient with normal renal function. *J Infect* 2003;46(4):286.
[124]. Debruyne H, Portzky M, Van den Eynde F, Audenaert K. Cotard's syndrome: a review. *Curr Psychiatry Rep* 2009;11(3):197-202.
[125]. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976;16(1):31-41.
[126]. Levey AS, Stevens LA. Estimating GFR using the CKD Epidemiology Collaboration (CKD-EPI) creatinine equation: more accurate GFR estimates, lower CKD prevalence estimates, and better risk predictions. *Am J Kidney Dis* 2010;55(4):622-7.
[127]. Braun JS. Delir wahrend der oralen Therapie eines Herpes zoster mit Aciclovir. Kasuistik und Kurzubersicht uber zentralnervose Nebenwirkungen von Aciclovir. *Nervenarzt* 1998;69(11):1015-8.
[128]. Adair JC, Gold M, Bond RE. Acyclovir neurotoxicity: clinical experience and review of the literature. *South Med J* 1994;28(4):1227-31.
[129]. Krieble BF, Rudy DW, Glick MR, Clayman MD. Case report: acyclovir neurotoxicity and nephrotoxicity--the role for hemodialysis. *Am J Med Sci* 1993;Suppl 1:36-9.
[130]. Mataix AL, Duarte J, Revuelta K et al. Oral acyclovir and neurologic adverse effects in endstage renal disease. *Ann Pharmacother* 1994;28:961-2.
[131]. Mahad D, Helldén A, Jarvis J, Mitra D, Gholkar A, Chinnery P. Aciclovir induced posterior leukoencephalopathy. *J Neurol Neurosurg Psychiatry* 2005;76(9):1308-9.
[132]. Heye N, Terstegge K, Gosztonyi G. Vasculitic, hypoxic-ischemic leukoencephalopathy. *Clin Neurol Neurosurg* 1994;96(2):156-60.
[133]. Lamy C, Oppenheim C, Meder JF, Mas JL. Neuroimaging in posterior reversible encephalopathy syndrome. *J Neuroimaging* 2004;14(2):89-96.
[134]. Smith J, Weller S, Johnson B, Nicotera J, Luther JM, Haas D. Pharmacokinetics of acyclovir and its metabolites in cerebrospinal fluid and systemic circulation after administration of high-dose valacyclovir in subjects with normal and impaired renal function. *Antimicrob Agents Chemother* 2010;54(3):1146-51.
[135]. Löscher W, Potschka H. Role of drug efflux transporters in the brain for drug disposition and treatment of brain diseases. *Prog Neurobiol* 2005;76(1):22-76.
[136]. Ohtsuki S, Asaba H, Takanaga H et al. Role of blood-brain barrier organic anion transporter 3 (OAT3) in the efflux of indoxyl sulfate, a uremic toxin: its involvement in neurotransmitter metabolite clearance from the brain. *J Neurochem* 2002;83(1):57-66.
[137]. Cotard J. Du délire hypochondriaque dans une forme grave de la méleancolie anxieuse. *Ann Med Psychol (Paris)* 1880;4:168-74.
[138]. Cotard J. Du délire des negations., Paris, 1882, 4: 152-282. *Archives de neurologie* 1882;4:152-282.

[139]. Pearn J, Gardner-Thorpe C. Jules Cotard (1840-1889): his life and the unique syndrome which bears his name. *Neurology* 2002;58(9):1400-3.
[140]. Asahi T, Tsutsui M, Wakasugi M et al. Valacyclovir neurotoxicity: clinical experience and review of the literature. *Eur J Neurol* 2009;16(4):457-60.
[141]. Caliyurt O, Vardar E, Tuglu C. Cotard's syndrome with schizophreniform disorder can be successfully treated with electroconvulsive therapy: case report. *J Psychiatry Neurosci* 2004;29(2):138-41.
[142]. Helldén A, Bergman U, von Euler M, Hentschke M, Odar-Cederlöf I, Ohlén G. Adverse drug reactions and impaired renal function in elderly patients admitted to the emergency department: a retrospective study. *Drugs Aging* 2009;26(7):595-606.
[143]. Pusateri DW, Muder RR. Fever, pulmonary infiltrates, and pleural effusion following acyclovir therapy for herpes zoster ophthalmicus. *Chest* 1990;98(3):754-6.
[144]. Almond MK, Fan S, Dhillon S, Pollock AM, Raftery MJ. Avoiding acyclovir neurotoxicity in patients with chronic renal failure undergoing haemodialysis. *Nephron* 1995;69(4):428-32.
[145]. Stathoulopoulou F, Almond MK, Dhillon S, Raftery MJ. Clinical pharmacokinetics of oral acyclovir in patients on continuous ambulatory peritoneal dialysis. *Nephron* 1996;74(2):337-41.
[146]. Togawa T, Furukubo T, Satoh M et al. Association between acyclovir metabolite CMMG and neuropsychiatric adverse reactions during acyclovir treatment in dialysis patients. *Japanese journal of pharmaceutical health care and sciences* 2007;33(7):585-90.
[147]. van Berlo-van de Laar IR, Goet ER, Helldén A, Sluiter HE. [Adverse effect of valaciclovir in disturbed kidney function]. *Ned Tijdschr Geneeskd* 2009;153(15):706-9.
[148]. van Kan HJM, de Jonge E, Bijleveld YA, Lie-A-Huen L, Beijnen JH. Viral encephalitis masking acyclovir neurotoxicity? A case report. *Netherlands Journal of Critical Care* 2009;13(2):96-8.