# Pharmacokinetics of Antispastic Drugs

## A study on baclofen and dantrolene

Eveline W. Wuis

## Aan de geïnteresseerde leek

Een geneesmiddel neem je in, omdat je er een bepaalde werking van verwacht. Anders gezegd, een geneesmiddel wordt geacht wat met het (zieke) lichaam te doen, bij voorkeur beter maken. Dit zal niemand onbekend in de oren klinken. Dat het lichaam ook wat met het geneesmiddel doet, is mogelijk minder bekend. Toch is dit heel voor de hand liggend. Iedereen die wel eens een 'aspirientje' heeft ingenomen, weet dat het even duurt voordat het effect merkbaar is en dat het na een paar uur weer is uitgewerkt. Hoe komt dat? Het lichaam zorgt ervoor, dat een geneesmiddel met een bepaalde snelheid wordt opgenomen in het bloed, wordt vervoerd, o.a. naar de plaats van werking, en na enige tijd, al dan niet in zijn oorspronkelijke vorm, weer wordt uitgescheiden. Door het prikken van bloed en soms ook het verzamelen van urine en ander materiaal, bijvoorbeeld speeksel, probeert men erachter te komen wat er precies in de tijd met het geneesmiddel gebeurt. Dit nu is het werkterrein van de farmacokineticus. Veelal beslaat dit ook het ontwikkelen van de chemische analysemethodes die nodig zijn om de concentratie aan geneesmiddel in het monstermateriaal te kunnen meten.

De geneesmiddelen die in dit proefschrift worden beschreven, baclofen en dantroleen, worden gebruikt door mensen van wie de spieren spastisch zijn ten gevolge van een aandoening in het centrale zenuwstelsel. Het zijn zogenaamde antispastische middelen. Baclofen is een eenvoudige chemische verbinding zoals is te zien in figuur 6 van het eerste hoofdstuk. Het is een afgeleide van gamma aminoboterzuur, een bepaald soort aminozuur dat normaal in het lichaam voorkomt. In vergelijking met de lichaamseigen voorraad aan aminozuren is de hoeveelheid baclofen die patienten krijgen toegediend maar klein. Het trachten terug te vinden van dit kleine beetje baclofen in bloed-en urinemonsters heeft veel weg van het zoeken naar een speld in een hooiberg, of, erger, naar een onzichtbare speld in een onzichtbare hooiberg. Aminozuren, en daarmee ook de afgeleide verbinding baclofen, kunnen namelijk niet 'zomaar' worden gemeten. Ze moeten eerst op de een of andere manier zichtbaar worden gemaakt. In dit proefschrift is gekozen voor koppeling met het 'OPA'-reagens, waardoor verbindingen ontstaan

die de eigenschap hebben fluorescentielicht uit te zenden. Zowel baclofen als de aminozuren kan men nu 'zien'. Om ze van elkaar te scheiden is een techniek gebruikt die kortweg HPLC wordt genoemd, oftewel hoge-drukvloeistofchromatografie (op zijn Engels afgekort). Bij deze techniek wordt in principe de onderkant van een stuk filtreerpapier in een bodempje vloeistof gehangen. Zit er op het filtreerpapier een mengsel van stoffen, dan zal de vloeistof die door het filtreerpapier wordt opgezogen het mengsel uit elkaar kunnen trekken, mits men de juiste vloeistof of vloeistoffen heeft gekozen. Door met hoge druk te werken gaat het allemaal wat sneller.

Uit de proeven bij dieren en mensen kon worden geconcludeerd dat de nier een belangrijke rol speelt bij het verwijderen van baclofen uit het lichaam. Baclofen bleek echter toch een ingewikkelder verbinding dan aanvankelijk werd aangenomen. Baclofenmoleculen kunnen namelijk in twee vormen voorkomen, een linksdraaiende en een rechtsdraaiende vorm (vergelijkbaar met melkzuur in yoghurt). De linksdraaiende en rechtsdraaiende moleculen zijn elkaars spiegelbeeld en zijn weergegeven in figuur 1 van hoofdstuk 7. Slechts de linksdraaiende vorm is werkzaam als antispastisch middel, de andere vorm is overbodige ballast. Helaas is het zo dat alle patiënten worden behandeld met een mengsel van deze twee vormen, waarbij een tablet voor de helft uit de onwerkzame vorm bestaat. In het lichaam kan deze 50-50 verhouding echter veranderen, zoals de experimenten met proefdieren aannemelijk maken. Het is dus zaak om bij de chemische analyse beide vormen van elkaar te kunnen onderscheiden. Dit alles betekent dat in de eerder aangehaalde hooiberg in feite twee spelden, die elkaars spiegelbeeld zijn, zaten verborgen. Het gescheiden meten van 'spiegelbeeldmoleculen' is vrij gecompliceerd. In dit proefschrift wordt een methode beschreven die als basis kan dienen voor verder onderzoek. Het verschijnsel van 50% overbodige ballast is trouwens niet voorbehouden aan baclofen. Bij een kwart van alle geneesmiddelen zoals we die nu kennen, heeft men met deze problematiek te maken. Pas de laatste jaren is hiervoor meer (publieke) belangstelling gekomen (De Volkskrant, zaterdag 27 januari 1990).

Dantroleen is een heel ander soort antispastisch middel dan baclofen. Het valt in het lichaam uiteen in verschillende stoffen, metabolieten

genaamd. Een aantal van deze stoffen is te zien in figuur 5 van hoofdstuk 1. Aangezien de hoeveelheid onveranderd dantroleen die door het lichaam wordt uitgescheiden minimaal is, kun je je bij farmacokinetisch onderzoek niet beperken tot het meten van alleen dantroleen, de zogenaamde moederverbinding. Dat is in feite farmacokinetiek op de 'vierkante millimeter' bedrijven. Gelukkig bracht ook hier de HPLC uitkomst. Uit de verschillende proeven kon worden geconcludeerd, dat de zogeheten hydroxy-metaboliet, waaraan ook enige antispastische activiteit wordt toegeschreven, in de urine (mens) of de gal (hond) terechtkomt. Het is echter zeer waarschijnlijk dat er nog meer metabolieten worden gevormd dan de eerder genoemde figuur suggereert. Wij vonden bijvoorbeeld een brokstuk van het in tweeen gesplitste molecuul in de urine.

Om terug te keren naar het begin: als een geneesmiddel niet het effect heeft wat men ervan verwacht, dan kan dit komen doordat het onvoldoende door het lichaam wordt opgenomen. Als dat zo is, zal de bloedconcentratie van het geneesmiddel, de zogenaamde bloedspiegel, te laag zijn. De arts kan de dosis ophogen, waardoor het effect alsnog optreedt. De dosis aanpassen aan de hand van bloedspiegels gebeurt soms ook als het vaststellen van het gewenste effect niet zo eenvoudig is. Nu is het meten van spasticiteit erg moeilijk. Als het bepalen van bloedconcentraties van antispastische middelen inderdaad kan bijdragen aan het vinden van de juiste dosis, is in de praktijk al veel gewonnen. In dit proefschrift wordt beschreven hoe het effect van antispastische middelen bij een bepaalde groep van patienten is te meten. Door tegelijkertijd bloedmonsters te nemen is in principe het verband tussen gewenst effect en bloedspiegel vast te stellen.

Nijmegen, 15 januari 1991 Eveline W. Wuis

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een wetenschappelijke proeve op het gebied van de geneeskunde en tandheelkunde in het bijzonder de geneeskunde

PROEFSCHRIFT

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geboren op 22 juli 1948
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Promotor: Prof. Dr. E. van der Kleijn

Co-promotor: Dr. T.B. Vree

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## Preface

The following clinical question underlies the work presented in this thesis: is it possible to guide dosage control of antispastic drugs in individual patients by measuring the drug plasma concentration? When the clinical effect of a drug is hard to evaluate, this approach to chronic drug therapy has previously proved to be successful. A thesis describing this method of regulating drug therapy for epilepsy has been written by Schobben (1). Whereas the epilepsy study could build upon abundant pre-existing pharmacokinetic knowledge, therapeutic drug monitoring in spasticity appeared to be a terra incognita.

The drugs most widely used for patients with spasticity are baclofen and dantrolene. For a meaningful interpretation of single-point plasma level determinations, which is usually one of the constraints of the clinical setting, knowledge of the pharmacokinetics of the drugs must be available. Although both baclofen and dantrolene have been in use since 1967, pharmacokinetic data are still scarce. The lack of sufficiently sensitive and specific analytical methods for pharmacokinetic purposes partially explains this. Moreover, the studies that are available have mainly focused on parent drug measurements in plasma, ignoring the information that can be obtained from metabolite and urine measurements.

An important aspect of the present study was therefore to develop chemical analyses suitable for pharmacokinetic studies for the quantitative determination of these drugs and their metabolites in biological fluids. An investigation of an enantioselective assay for baclofen, which is marketed as the racemate, was also undertaken.

The pharmacokinetics of racemic baclofen in man were studied both after a single oral dose and during chronic treatment. Because of the reported toxicity in patients with renal function impairment, influence of the renal function on the kinetics was anticipated. Lack of toxicity data on the enantiomers of baclofen prompted the use of the dog to study the pharmacokinetic behaviour of the isomers when individually administered.

The complexity of its disposition led to animal studies with dantrolene. Whole-body autoradiography in a small primate, the marmoset monkey, was chosen to detect regional presence of the $^{14}C$-labelled compound. Parent drug and metabolite kinetics were studied in the dog following intravenous administration of dantrolene and 5-hydroxydantrolene. Considering their physico-chemical properties, these compounds might undergo excretion in the bile. The pharmacokinetic behaviour of dantrolene in man was only briefly encountered

A clinical pharmacokinetic study was undertaken in mentally retarded patients suffering from cerebral palsy. The particular patient population was chosen because they were being treated with antispastic drugs without adequate control of the clinical benefit. Since methods to measure response suited to the patients were not available, the development of simple tests became another aspect of this study.

The aims of the present investigations can be summarised as follows:

1. to develop analytical methods for the quantitative determination of baclofen, dantrolene, and their metabolites, and

2. to apply these methods for the study of selected pharmacokinetic aspects of the drugs in animals, human volunteers, and patients.

Reference

1. Schobben AFAM. Pharmacokinetics and therapeutics in epilepsy. Nijmegen: Catholic University, 1979. Dissertation.

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# SECTION I

# LITERATURE REVIEW

# Chapter 1

# SPASTICITY AND DRUG THERAPY

# SPASTICITY AND DRUG THERAPY[1]

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**Abstract**

An overview is presented of pathophysiology, classification and measurement of spasticity and of its treatment, especially with dantrolene and baclofen. In spasticity, the balance between excitatory and inhibitory neurotransmitters in the central nervous system is impaired by mechanisms that are for the greater part unknown. Spasticity includes various disorders of motor control, and classification is needed for a meaningful evaluation of antispastic therapy. Cerebral palsy is a specific disorder, sometimes also called spasticity. Measurement of spasticity is complicated and should include signs characteristic of spasticity and parameters for clinical improvement. Dantrolene and baclofen have established their place in the treatment of spastic disorders, but a preference for either drug is hard to give. For tizanidine it is still too early to determine its place in therapy. Dantrolene is a direct acting muscle relaxant which should be avoided in patients with pre-existing liver damage. Its mechanism of metabolism and excretion is for the greater part unknown. The $GABA_B$ agonist baclofen is a centrally acting muscle relaxant. In patients with impaired renal function the dose should be reduced. Abrupt withdrawal carries the risk of unwanted reactions. The R(-)-enantiomer has proved to be the active isomer. This means that human trials need reappraisal, especially those relating to the pharmacokinetics of the racemate.

**Key words:** Baclofen; Cerebral palsy; Dantrolene; Drug therapy; Muscle relaxants; Spasticity; Tizanidine

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[1]Pharm Weekbl [Sci] 1987; 9: 249-60.

## Introduction

What is spasticity? Spasticity is not a disease but a disorder of motor control observed in a number of neurological conditions (Table I) (1). The word is derived from the Greek word 'σπαστικος' which means 'he that draws or pulls' (patients with spasticity are often subject to sudden and violent muscular contractions). Depending upon the context, the term spasticity has several varieties of meaning. In the narrow sense, spasticity is a mere clinical sign referring to a specific type of increased muscle tone felt during passive movement. The increased muscle tone of spasticity is a function of the velocity with which the muscle is stretched, differentiating it from the hypertonia of Parkinsonism and other forms of muscle hypertonia.

The whole syndrome of spasticity, however, is much more complex and is characterized by a group of negative and positive signs, originally described by Jackson (Fig. 1) (2). Negative signs are weakness and loss of dexterity, while positive signs include increased muscle tone ('spasticity'), hyperreflexia and flexor spasms. In this sense, spasticity is nowadays defined according to Lance as 'a motor disorder characterized by a velocity-dependent increase in tonic stretch reflexes ('muscle tone') with exaggerated tendon jerks, resulting from

TABLE I

Most important neurological conditions in which spasticity may occur (reprinted with permission from ref. 1)

| Aetiology | Incidence |
|---|---|
| Trauma | + + |
| Cerebrovascular insult (apoplexy) | + + + |
| Autoimmune disease (multiple sclerosis) | + + + |
| Tumours | + |
| Infections, intoxications (Pb, Hg, Mg) | + |
| Dysontogenesis (cerebral palsy, Arnold-Chiari syndrome) | + + |

hyperexcitability of the stretch reflex, as one component of the upper-motor neuron syndrome' (2). In this definition not all signs are included. The syndrome spasticity may develop following a lesion in descending motor pathways in the spinal cord or at a supraspinal level. Depending upon the localization and the extent of the lesion, the clinical appearance can be different. Sometimes, paralysis of one or more limbs is present.

Of the neurological conditions given in Table I, cerebral palsy is also referred to as 'spasticity'. The striking presence of the signs of spasticity in the majority of cerebral palsy cases, has led to this confusing interchange of terms. Cerebral palsy or 'spasticity' in this specific sense is defined as 'a collection of nonprogressive neuromotor disorders of central origin that become manifest early in life and are not the result of a recognized cerebral malformation' (3). In the United States, cerebral palsy affects about one out of every fivehundred school-age children, half of them with mental retardation, a quarter with seizure disorders. In the Netherlands, the prevalence in the total population is about one per thousand (4).

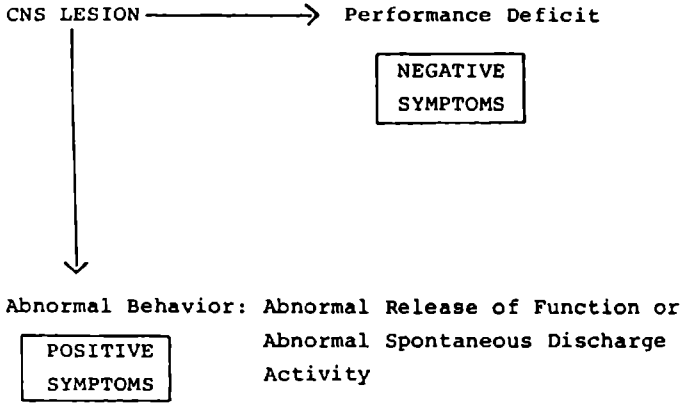



FIGURE 1
'Jacksonian' negative and positive 'symptoms' observed in spasticity (reprinted with permission from ref. 2)

The stretch-reflex arc plays an important role in muscle control (Fig. 2) (5). Within skeletal muscles, muscle spindles are located which transmit information about changes in muscle length along Ia fibres to the spinal cord. Via monosynaptic and polysynaptic connections impulses are transmitted back to the muscles through so-called extrafusal efferents of alpha motoneurons. Fusimotor efferents of gamma motoneurons adjust the sensitivity of the spindles. The most simple stretch reflex is the tendon jerk, which is monosynaptic. In normal harmonious movement, more complicated polysynaptic stretch reflexes and other spinal reflexes are involved, which are further controlled at spinal and supraspinal levels.

Acetylcholine acts as an excitatory transmitter at all neuromuscular junctions. Within the spinal cord, a number of excitatory and inhibitory neurotransmitters play a role. Glutamate and aspartate e.g., are responsible for excitation, glycine and gamma-amino butyric acid (GABA)

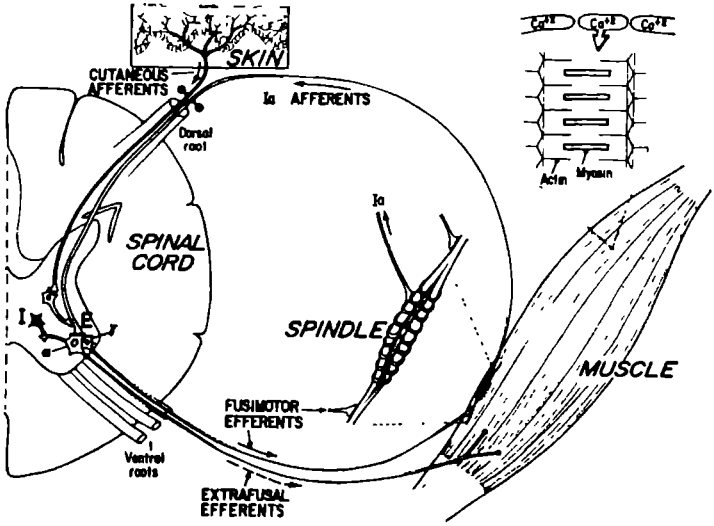



FIGURE 2

The stretch-reflex arc (for explanation see text). E = excitatory presynaptic ending; I = inhibitory interneuron, $\alpha$ = alpha motoneuron, $\gamma$ = gamma motoneuron (reprinted with permission from ref. 5)

TABLE II

Several classifications of spasticity

| Classification criteria | Class name | Brief characterization | Major signs |
|---|---|---|---|
| Site of lesion | spinal spasticity | lesion within the spinal cord | tendency to flexion |
| | cerebral spasticity | lesion at a supraspinal level | motor handicap ranges from purely spastic to severely dyskinetic*, tendency to weakness |
| Cryotest | alpha spasticity | hyperactivity of alpha motoneurons | increased muscle tone on local cooling |
| | gamma spasticity | hyperactivity of gamma motoneurons | decreased muscle tone on local cooling |
| Neurophysiological tests | | hyperactivity of alpha motoneurons | clinically similar patients may have different pathophysiology |
| | | impaired GABA-ergic presynaptic inhibition | |
| | | impaired (glycinergic) interneuronal inhibition | |

* Involuntary movement at rest with changing muscle tone. For further explanation see text.

for inhibition, the latter selectively at presynaptic nerve endings.

For increased muscle tone and hyperreflexia to develop, the stretch-reflex arc must be intact. Several mechanisms underlying these positive signs of spasticity have recently been identified by neurophysiological testing of spinal cord functions (6). In patients with spasticity, the activity of alpha motoneurons is higher than in normal volunteers. Of the inhibitory mechanisms, GABA-mediated presynaptic inhibition on Ia fibres and a specific type of probably glycine-mediated interneuronal inhibition may be impaired, the preponderance of either varying considerably between patients. Progress in clarifying other underlying mechanisms, especially on the supraspinal level, is being complicated by the lack of animal models resembling human spasticity.

## Classification

As spasticity includes various disorders of motor control, a classification will contribute to a better understanding of prognosis and management. Considering the above, it will not come as a surprise that classification criteria are poor. A very simple clinical criterion can be the severity of the disorder. Pedersen gives a clear description of what he calls mild, moderately severe, and severe spasticity (7). Terms commonly found in the literature, are given in Table II. Depending upon the site of the lesion, a distinction is made between spinal and cerebral spasticity. This anatomical classification is clinically useful. In spinal spasticity following a period of shock, the response to all afferent inputs is exaggerated; patients tend to develop a flexed posture. In cerebral spasticity the generalized reflex hyperexcitability is usually less severe; besides increased muscle tone, weakness may present as a major problem.

In Scandinavian literature, a simple test can be found to distinguish between hyperexcitability of alpha and gamma motoneurons (8). This so-called cryotest, which is based upon the effect of local cooling on the spastic muscle, should differentiate between the two and has been used as a rough criterion to select a specific drug treatment. Findings with specialized interneuronal techniques, however, do not support the con-

cept of hyperexcitability of the gamma system (6) If this is true, then the theory behind the cryotest needs reappraisal.

The earlier indicated neurophysiological tests which distinguish between different spinal cord abnormalities, enable the development of a new type of pathophysiologically based classification (6). This classification will become of importance for drug therapy, the more so as clinically similar patients may have different pathophysiologies As long as this neurophysiologic testing is no current practice, at least a careful description of the disorder should be a prerequisite for a meaningful evaluation of an antispastic therapy. This applies also to the various motor forms of cerebral palsy, which can clinically be classified as spastic cerebral palsy, dyskinetic cerebral palsy (characterized by involuntary movement at rest with changing muscle tone) and mixed forms (9).

## Measurement

Measurement of spasticity is needed to be able to document its severity and to record changes in relation to a given treatment Quantitative methods of measurement are especially important when the aim is a reduction in tone and not its total abolishment. This is as true for the individual patient who wants to benefit from his treatment, as for selected groups of patients undergoing clinical trials with new drugs.

In the clinical evaluation of spasticity, resistance to passive movement is still frequently recorded according to Ashworth (1964)· the examiner moves the spastic limb and expresses his subjective impresssion in a simple five-grade scale (10). To obtain a less subjective and better quantifiable measure of tone, many biochemical apparatus have been developed. An example is the relative equivalent damping (RED) apparatus of Kwee, used by Meyler to study the effectiveness of dantrolene (11). The RED-device measures the force required from the examiner to move the spastic limb against the pathological resistance.

Measurement of tone is usually part of a more elaborate neurological rating system, of which many have been developed. In a comparison of five of these rating systems in the same group of patients with multiple sclerosis, Grynderup gives some recommendations to achieve the max-

imum benefit (12). He concluded that the effect of a therapy is best measured with a relatively large number of grades, which would favour the so-called Ry-system for this purpose.

For information about the quality of life following a given treatment, assessment of activities of daily living (ADL) is better suited than a neurological examination. A still useful test is the Barthel Index: 'A simple index of independence to score the ability of a patient with a neuromuscular or musculoskeletal disorder to care for himself, and by repeating the test periodically, to assess his improvement' (13). Compared with two other ADL indices in a group of stroke survivors, the Barthel Index had certain advantages: 'Completeness, sensitivity to change, amenability to statistical manipulation, and a greater familiarity due to more widespread use' (14). The Barthel Index has been used in a long-term study on baclofen in patients with multiple sclerosis (2).

A major function frequently used as a test parameter in spasticity, is gait. Methods of analysis range from clinical observation, preferably recorded on film or otherwise, to sophisticated electrophysiological tests. A rating scale for gait analysis in children with spastic cerebral palsy was developed by Postema (15). Comparison of video recordings before and after dantrolene treatment was part of his study on the effectiveness of this drug. He also developed the rapid repetitive movement (RRM) test for quantitative measurement of voluntary motor control, which measures irregularity, slowness and limited freedom of movement.

In the judgement of treatment programmes it is important to realize what is being measured: functional improvement does not necessarily mean treatment of spasticity. The latter preferably requires quantitative electrophysiological analyses, the next step in the tests earlier referred to (6,16). As each test has its limitations, a combination of methods is usually necessary. For the measurement of the dyskinetic and mixed forms of cerebral palsy, hardly any test is available. The electronic measurement of limb movement and function, developed by Brown to construct feeding devices for patients with dyskinetic cerebral palsy, might also prove useful in testing the effectiveness of a given treatment (17). Still other demands are made by cerebral palsy patients with mental retardation. In addition to the mental retardation, frequently

occurring disturbances in behaviour, consciousness, vision, hearing and speech further limit the possibilities of measurement. A simple functional motor rehabilitation scale for moderately retarded children is given by Banham (18).

## Management

Not all forms of spasticity require management. In mild cases, the discomfort may be so slight that the disadvantages of treatment become predominant. In patients with accompanying paralysis, treatment may lead to loss of their ability to remain in an upright position. A careful consideration of the pros and cons should precede any management plan, especially in the case of destructive methods.

Generally, treatment is started with physical therapy. Various programmes have been developed which are aimed at avoiding the shortening of tendons and muscles, preventing contractures, and improving voluntary power (7). For patients with cerebral palsy, specific training systems are available (17,19). Drug therapy with muscle relaxants is usually given in addition to exercise programmes. Only a very limited number of drugs has proved to be clinically useful in the management of spasticity (see below). For the dyskinetic form of cerebral palsy no specific drugs are available.

Severe spasticity may require chemical destruction or surgical measures (7). Chemical destruction is performed with sclerosing agents such as alcohol and phenol in various dilutions. Injections at different sites along the stretch-reflex arc have given good results, but poor selectivity of action and recurrence of symptoms limit their use. In selected patients, improvement can be obtained with orthopedic surgical procedures, such as elongation or sectioning of tendons. Dissection of afferent or efferent fibres, stereotactic brain surgery and other neurosurgical procedures are a last resort for patients not controllable by any other means and presently rarely used.

Pharmacologically, spasticity can be interfered with by interrupting the stretch-reflex arc at a number of sites (Fig. 2). Drugs have been developed which act at the neuromuscular junction (curare-like agents), at spinal or supraspinal levels (centrally acting muscle relaxants), or on the muscle itself (dantrolene).

In Table III drugs with muscle relaxant properties are given in a chronological order. Curare paralyses skeletal muscles by blocking acetylcholine receptors at the neuromuscular junction. But because of the elimination of voluntary power and the involvement of respiratory muscles, curare-like agents are no longer employed outside the operating room or the intensive care unit (7). In 1910, with the discovery of 3-phenoxy-1,2-propanediol, a substance was found which caused a reversible paralysis in animals (20). Its derivative 3-o-toloxy-1,2-propanediol, or mephenesin, became the prototype of a series of centrally acting muscle relaxants (21). The early compounds, however, were either too short-acting or lacking clinical effectiveness. The longer-acting carbamates meprobamate (2-methyl-2-propyl-1,3-propane-diol dicarbamate) and mephenoxalone (a cyclic carbamate) are clinically effective but too sedative to be of use in spasticity.

All benzodiazepines have muscle relaxant properties. Traditionally, diazepam is the benzodiazepine most often used for muscle relaxation. Being a so-called classical $GABA_A$-agonist it reinforces depressed presynaptic inhibition in the spinal cord (5,16). For long-term administration in spasticity, however, it is usually not the drug of choice, again because of sedation. For this purpose, baclofen has been the preferred centrally acting muscle relaxant over the past twenty years (see below). Recently, baclofen was joined by tizanidine Fig. 3).

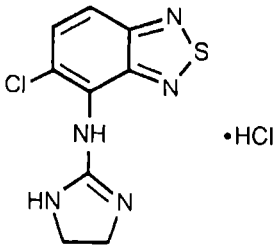



FIGURE 3
Structure of tizanidine, a new centrally acting muscle relaxant

TABLE III

Historical review of major muscle relaxants

| Year of introduction | First compound | Chemical class | Some currently available compounds | Ref*-erence |
|---|---|---|---|---|
| <1900 | curare | curare-like agents | tubocurarine (Curarin®) | 7 |
| 1910 | 3-phenoxy-1,2-propanediol | glycerol ethers | | 20 |
| 1943 | benzimidazole | benzazole compounds | chlorzoxazone (Paraflex®) tizanidine**(Sirdalud®) | 20 |
| 1946 | mephenesin | (carbamates of) glycerol ethers | | 21 |
| 1954 | meprobamate | (cyclic) carbamates of glycols | mephenoxalone (Dorsiflex®) | 22 |
| 1961 | chlordiazepoxide | benzodiazepines | diazepam (Stesolid®, Valium®) | 23 |
| 1967 | baclofen | GABA derivatives | baclofen (Lioresal®) | 24 |
| 1967 | dantrolene | imidazolidinones | dantrolene (Dantrium®) | 28 |
| 1977 | tizanidine** | imidazoline derivatives | tizanidine**(Sirdalud®) | 25 |

* References on first compound.

** Can be classified either as benzazole or imidazoline derivative

Tizanidine can be classified as an imidazoline or as a new derivative of the benzazoles of which chlorzoxazone has been a rather ineffective example (20). Tizanidine interferes with polysynaptic reflexes in the spinal cord, possibly by inhibiting the release of the excitatory neurotransmitter aspartate (26). Its effect was comparable to baclofen in a number of trials, but more studies are needed before its place in the management of spasticity can be determined (26,27). The only drug interfering at the muscular level of the stretch-reflex arc, is dantrolene.

## Dantrolene

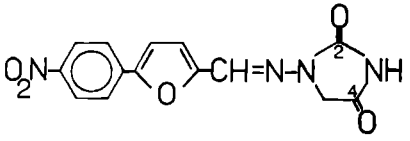
Dantrolene, 1-[[5-(p-nitrophenyl)-furfurylidene]-amino] imidazolidine-2,4-dione (Fig. 4), was the most active compound of a series of substituted 2,4-imidazolidinones (=hydantoins) synthesized by Snyder et al. (28). Till today, it has remained the only clinically useful imidazolidinone derivative for muscle relaxation in spasticity. Of the other imidazolidinones given in Figure 4, phenytoin has incidentally been used as co-medication in spastic disorders (29). The structural analogue nitrofurantoin is not a muscle relaxant, but has resemblance to dantrolene in chemical stability and side effects. Both compounds are susceptible to acidic cleavage of the azomethine bond, and may cause a similar type of liver and lung injury on prolonged use (30,31).

Clodanolene is an experimental compound in which the p-nitrophenyl group of dantrolene has been replaced by a 3,4-dichlorophenyl moiety. It has been tested in animal models of muscle relaxation and malignant hyperthermia (32,33). Malignant hyperthermia is a genetically determined disorder associated with a high mortality rate if left untreated. Dantrolene has been used very successfully in the treatment of this syndrome in addition to its use as an antispastic agent (34). Although clodanolene offered no clear advantage over dantrolene in the experimental models, the lack of the nitro-group makes it an interesting compound as intermediates of nitro reduction have been associated with hepatotoxicity (35). The newest offspring of the imidazolidinone tree is the class of the oxazolyl-2,4-imidazolidinones, in which the furan

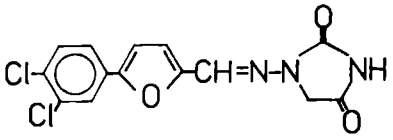
moiety has isosterically been replaced by oxazole (36). At present, these compounds are undergoing pharmacological evaluation.

MODE OF ACTION

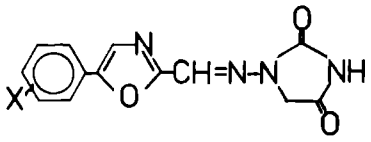
Dantrolene is a direct acting muscle relaxant. It acts intracellularly by affecting calcium flux across the sarcoplasmic reticulum of skeletal muscles. Its effect on muscle spindles is less clear (37). Cardiac and smooth muscle are not as sensitive to dantrolene as skele-



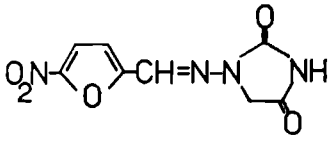
dantrolene



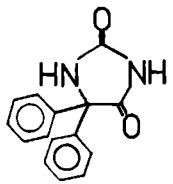
clodanolene



oxazolyl derivatives



nitrofurantoin



phenytoin

FIGURE 4
Substituted 2,4-imidazolidinones (=hydantoins)

tal muscle, probably because they are more dependent upon external calcium which is influenced by calcium entry blockers like verapamil (38). Whether dantrolene also exhibits calcium entry blocking properties is still controversial (39,40). Farquhar et al. offer some explanations for the minimal effect of dantrolene on respiration (41). From their experiments with rats, they propose three compensatory mechanisms contributing to maintenance of ventilation. Clinically, one should remain cautious, however, as two cases of depressed respiration following dantrolene administration have been reported in literature (42,43).

## BIOANALYSIS

In 1968, Hollifield and Conklin developed a spectrophotofluorimetric method for dantrolene in biological fluids, which was later modified to account for the presence of metabolites (44,45). For pharmacokinetic purposes the various high-pressure liquid chromatographic (HPLC) assays of a later date offer better specificity and sensitivity (46,47). Dantrolene plasma concentrations differ from concentrations in whole blood. This should be kept in mind when different studies are compared, e.g. effective 'levels' in malignant hyperthermia (48,49). Dantrolene and its metabolites are not stable under all conditions. The hydantoin ring can open in the presence of alkali, the azomethine bond is susceptible to acid (30,50). In the presence of enteric bacteria dantrolene degrades to 7-aminodantrolene of which the acetylated form is light sensitive (47,51).

## PHARMACOKINETICS

About the rate and extent of absorption of dantrolene little is yet known. No data on bioavailability have been reported. Mass balance studies are hampered by the aforementioned instability problems. Peak plasma concentrations have been reported to occur several hours after oral administration (11,46,52). In a group of cerebral palsy patients a poor correlation was found between daily oral dose and plasma concentration (53).

Distribution has been investigated with fluorescent techniques in mice. The highest concentrations were found in the intestine and in the liver (54). This agrees with an experiment with $^{14}C$-dantrolene in a monkey (Wuis EW, unpublished observations). No fluorescence nor radio-

activity was found in the brain In vitro binding of dantrolene to serum albumin, red blood cells, sarcoplasmic reticulum and hepatic endoplasmic reticulum has been demonstrated, the latter also in vivo (55-57). Binding to the cornea has been suggested to occur in a child and this is not contradictory to observations in the earlier mentioned monkey experiment (58). Dantrolene crosses the placenta as measured in two cases (59)

The proposed metabolic scheme of dantrolene is given in Figure 5 (60). It has been concluded from animal experiments that 5-hydroxydantrolene contributes to some extent to the effect of dantrolene (60, 61). Renal excretion in man accounts for 15 25% of an intravenous or oral dose, mainly as the 5-hydroxy compound (52,62). As biliary and faecal excretion were found to be negligible, the major route of elimination of dantrolene is still unknown (52,63). Elimination half-lives of dantrolene after oral administration show large interindividual variations with extreme values of 3 and 22 hours (11,52). In several stud-

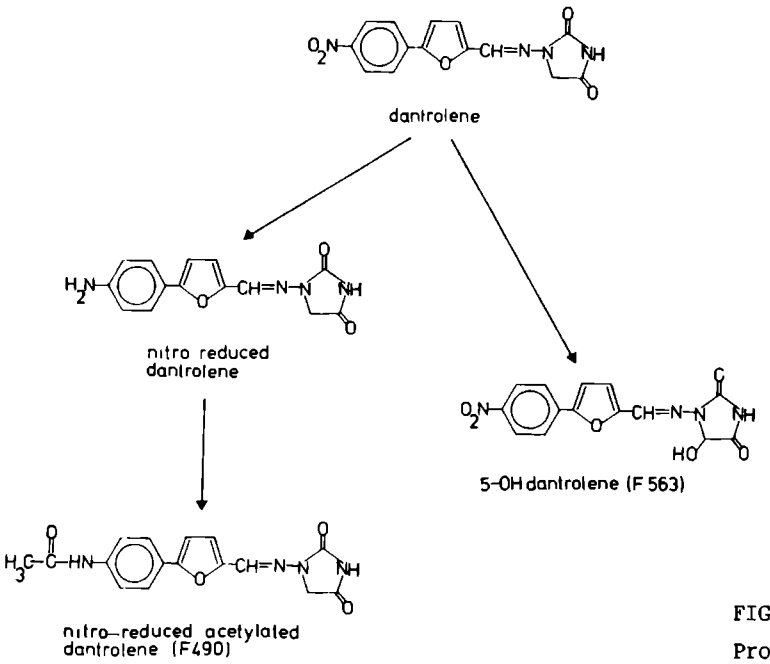



FIGURE 5
Proposed metabolism of dantrolene

ies a correlation was found between dantrolene plasma concentration and pharmacological effect, like depression of twitch tension. However, a correlation between plasma concentration and therapeutic effect in spasticity is less clear (11,15). As a minimum effective plasma concentration 0.3 mg/l has been suggested (11).

THERAPEUTIC USE

Many studies have been published on the use of dantrolene in spasticity. How to determine the benefit of treatments has been the most difficult part of all studies (see above). The peripheral site of action of dantrolene precludes electrophysiological testing of spinal cord functions to measure its effectiveness. The heterogeneity of the patients treated, even within a study, and the lack of objective quantitative methods of measurement, make the drawing of definite conclusions about its effectiveness for a specific type of spastic disorder a difficult task. In spasticity associated with multiple sclerosis dantrolene seems to be less effective than in other types of spas tic disorders. Nevertheless, its overall efficacy in patients with multiple sclerosis is comparable to that of baclofen (34).

In Table IV a summary is given of the results of controlled double-blind studies in children with cerebral palsy. In most studies subtle benefits were found, but these seldom warranted continuation of the drug after the trial period. In cerebral palsy, dantrolene has not been compared with baclofen. The results already obtained in 1973 by Chyatte et al. in patients with the dyskinetic form of cerebral palsy, ask for further studies (64). For an overview which also includes the use of dantrolene in malignant hyperthermia and related hypercatabolic disorders, one is further referred to the article by Ward et al. (34).

Hepatic injury has been the most feared side effect of dantrolene therapy. Fatal hepatitis has occurred in about 0.3% of patients. Since dosage recommendations for long-term treatment have been reduced to 200 mg per day, the risk of liver damage appears to have decreased. In patients with pre-existing liver damage, dantrolene is still contraindicated. Other side effects of dantrolene therapy are usually less severe (34).

TABLE IV

Summary of results of controlled, double-blind trials of dantrolene in patients with cerebral palsy

| | Number of patients | Dosage regimen | Duration of therapy* (weeks) | Effect (number of patients in brackets) | Classification of cerebral palsy | Age of patients (years) | Reference |
|---|---|---|---|---|---|---|---|
| Dantrolene versus placebo | 17 | dantrolene 20-400 mg/day<br>placebo | 4 | dantrolene > placebo (12)<br>dantrolene = placebo (5) | dyskinetic | 7 -38 | 64 |
| | 28 | dantrolene 12 $mg.kg^{-1}.d^{-1}$<br>placebo | 3 | dantrolene > placebo (5 )<br>dantrolene = placebo (23) | spastic | 1.5-12 | 65 |
| | 4 | dantrolene 1-10 $mg.kg^{-1}.d^{-1}$<br>placebo | 1.5 | dantrolene ≈ placebo | spastic | 9 -17 | 66 |
| | 23 | dantrolene 12 $mg.kg^{-1}.d^{-1}$<br>placebo | 1 | dantrolene ≥ placebo | spastic | 1.5-17 | 67 |
| | 20 | dantrolene 1-15 $mg.kg^{-1}.d^{-1}$<br>placebo | 6 | dantrolene = placebo | spastic | 4 -15 | 68 |
| Dantrolene versus diazepam | 22 | dantrolene ≤ 225 mg/day<br>diazepam ≤ 12 mg/day | 3 | dantrolene > diazepam (9)<br>dantrolene < diazepam (7)<br>dantrolene = diazepam (4) | spastic | 2 - 8 | 69 |

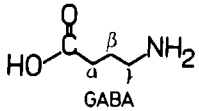
* On maximum dose only.

## Baclofen

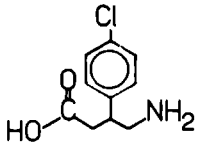
Baclofen, 4-amino-3-(p-chlorophenyl)butyric acid (Fig. 6), is the product of research directed at the development of a drug with GABA-ergic properties. Later it became known, after baclofen had already been introduced into therapy for spasticity, that it was not a true GABA agonist. Of the clinically administered racemate the R(-)-isomer (Fig. 7) is active at so-called $GABA_B$ receptors Other actions of baclofen, like its effect in tardive dyskinesia and schizophrenia, have been connected with the phenethylamine moiety of the molecule (Fig. 6) (70). At present, baclofen is the only drug in clinical use with $GABA_B$ agonistic properties.

### MODE OF ACTION

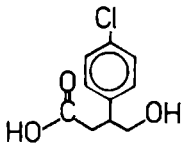
An overwhelming amount of literature has been published on baclofen and its proposed mode of action. The results of early publications in which the racemate was tested for GABA-ergic activity, are rather con-



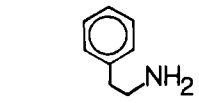
GABA



baclofen

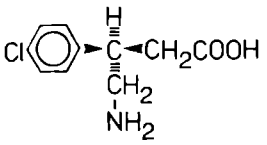


baclofen metabolite

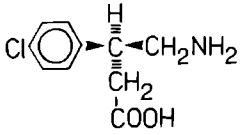


phenethylamine

FIGURE 6
Structural formulae of GABA, baclofen, its γ-hydroxy metabolite and phenethylamine



R(-)baclofen



S(+)baclofen

FIGURE 7
Enantiomers of baclofen

fusing. Some clearness was created when the enantiomers were tested separately in addition to the recognition of two types of GABA receptors: the classical bicuculline-sensitive $GABA_A$ receptors linked to chloride channels, and the newly discovered $GABA_B$ receptors which are probably linked to calcium channels (71). The main site of action of baclofen seems to be on presynaptic $GABA_B$ sites within the spinal cord, where it stereospecifically depresses the release of excitatory amino acids (72).

$GABA_B$ receptors have not only been found in the spinal cord, but also at supraspinal levels and even extraneuronally in the rabbit uterus (73). Their localization is not restricted to presynaptic nerve terminals; postsynaptic sites that are linked to potassium channels, may also be involved (74,75). Although benzodiazepine receptors have been linked with $GABA_A$ recognition sites, there is some evidence for the involvement of baclofen with benzodiazepine receptors, possibly mediated by the postsynaptic $GABA_B$ sites (76). The implications of these findings for the clinical effectiveness of baclofen are not yet clear.

Terrence et al. found structural similarities between baclofen and carbamazepine (77). This last observation leads into the discussion about baclofen and epilepsy. Contradictory data have been published so far. Recently, R(-)-baclofen was found to act as a convulsant in vivo in the rat (78). In earlier studies anticonvulsant activity had been found, at least in some models of epilepsy (79). Another controversy is the analgesic action of baclofen which has been demonstrated in animal studies (80,81). In man, analgesia with baclofen seems to be restricted to trigeminal pain (82, 83).

BIOANALYSIS

Baclofen can be determined in biological fluids either by gas chromatography or by HPLC (84-86). In general, extraction and derivatization procedures are needed for detection of the GABA derivative baclofen in the presence of endogenous amino acids. The above-mentioned methods do not selectively measure the two enantiomers. Some preliminary results of enantioselective assays have been published, but chiral recognition in biological fluids has not yet been attained (87,88). For the proposed metabolite of baclofen, 4-hydroxy-3-(p-chlorophenyl)butyric acid (Fig. 6), an HPLC assay was recently developed (86).

PHARMACOKINETICS

After intravenous and oral administration of $^{14}$C-labelled baclofen about 80% of radioactivity was recovered in the urine, mainly as unchanged drug (2,89). Food did not significantly alter the rate and extent of absorption (2,90). The time to reach peak plasma concentrations after an oral dose was 1-2 hours (91). $^{14}$C-labelled baclofen was used for distribution studies in the mouse and the rat. High concentrations were found in the liver and the kidneys, low concentrations in the brain and the spinal cord (2,89). In man, binding of baclofen to plasma proteins is low, about 20-30% (2,90). Concentrations in the cerebrospinal fluid were usually ten times lower than their corresponding plasma values (92). Only 0.1% of a single oral dose of 20 mg was found in breast milk (93).

About 70% of baclofen is excreted renally as unchanged drug. Diminished renal function requires dosage reduction (94). Ibuprofen-induced renal insufficiency has led to baclofen toxicity (95). Whether diminished creatinine clearance in the elderly is also responsible for the greater incidence of side effects in this population, has not entirely been ruled out (96). One pharmacologically inactive metabolite has been found which accounts for only a very small fraction of the administered dose (Fig. 6) (2,89). Elimination half-lives range between 2 and 6 hours (2,89). The prolonged elimination half-life (30-35 hours) after massive overdoses as found by Lipscomb et al. and Ghose et al. was not confirmed in a later case (97-99). Very limited data are available on the relationship between plasma concentration and effect. Reduction in

spasticity has been observed with concentrations between 0.1 and 0 6 mg/l (92).

All of the above-mentioned studies were carried out with the racemic drug without the possibility of enantioselective analysis. This means that not much weight can be attached to the pharmacokinetic data presented. An enantioselective assay is needed to enable measurement of the 'true' parameters.

THERAPEUTIC USE

Like dantrolene, baclofen has established its place in the treatment of spasticity. Again, evaluation of the effect has been the most difficult part of all studies (7,17). In one study, the mode of action of baclofen was tested with electrophysiological analysis of spinal cord functions. However, this study, which also included tizanidine and diazepam, did not yet assess the clinical benefit of treatment (16). An overview of the use of baclofen in cerebral palsy is given in Table V. In most studies baclofen had some effect, mainly in patients with the spastic form of cerebral palsy. The present data do not allow to choose for either baclofen or dantrolene in a specific type of spastic disorder. For more information on the use of baclofen, the proceedings of three symposia on baclofen and spasticity held in the past fifteen years are available (2,17,102).

Intrathecal application of baclofen proved to be effective in a small number of patients with severe spasticity unresponsive to oral baclofen (103). A case of reversible coma following intrathecal baclofen, indicates that this mode of administration warrants extreme caution (104). Long-term administration carries the risk of seizures, hallucinations, and dyskinesias on abrupt withdrawal (105,106). To circumvent these complications, a dosage reduction of 5-10 mg per week is recommended (107).

## Conclusions

If one wants to treat spasticity, it is necessary to make clear what spasticity is. In recent literature, the definition of Lance is generally agreed upon (2). As spasticity is not a single entity, a classifi-

TABLE V

Summary of results of placebo-controlled, double-blind trials of baclofen in patients with cerebral palsy

| Number of patients | Dosage regimen | Duration of therapy* (weeks) | Effect | Classification of cerebral palsy (number of patients in brackets) | Age of patients (years) | Reference |
|---|---|---|---|---|---|---|
| 35 | 45 mg/day | 2 | baclofen > placebo | spastic | 3-61 | 2,pp41-9 |
| 20 | 30-60 mg/day | 2 | baclofen > placebo | spastic (17)<br>mixed (3) | 2-16 | 17,pp16-22 |
| 18 | 2 $mg.kg^{-1}.d^{-1}$ | 1 | baclofen = placebo | spastic (12)<br>mixed (6) | 7-16 | 100 |
| 36 | 30-70 mg/day | 4 | baclofen $\geq$ placebo | spastic (21)<br>mixed (15) | 2-17 | 101 |

* On maximum dose only.

cation will aid in the judgement of the different treatment programmes. A classification based upon the underlying pathophysiological mechanisms offers the best perspectives for treatment with drugs As long as the required electrophysiological tests are not universally available, a careful description of the disorder is a prerequisite for meaningful comparisons. Which method should be selected or developed for the measurement of spasticity is dependent upon the primary aim of the treatment. A combination of methods is usually necessary as each method has its limitations.

Muscle relaxants can be useful in the management of spasticity, but the advantages and disadvantages should be carefully considered. If drug therapy is decided upon, evaluation of the expected effect is of paramount importance in preventing unnecessary chronic drug consumption. Of the many drugs that have been developed as muscle relaxants, only a few have survived as clinically useful. For the past twenty years only baclofen and dantrolene have been widely used, each with its own limitations. New developments are to be expected with the use of electrophysiological tests, which can discriminate between the various pathophysiological abnormalities of spasticity. Matching the pharmacological profile of muscle relaxants with the pathophysiological profile of spasticity may eventually lead to a better treatment.

Cerebral palsy is not synonymous with spasticity, but it is a specific disorder, often with signs of spasticity. Little is known about the measurement and management of the dyskinetic and mixed forms of cerebral palsy.

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# SECTION II

# ANALYTICAL PROCEDURES

Chapter 2

# HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF BACLOFEN IN PLASMA AND URINE OF MAN AFTER PRECOLUMN EXTRACTION AND DERIVATIZATION WITH o-PHTHALDIALDEHYDE

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# HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF BACLOFEN IN PLASMA AND URINE OF MAN AFTER PRECOLUMN EXTRACTION AND DERIVATIZATION WITH *o*-PHTHALDIALDEHYDE

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## SUMMARY

A reversed phase high performance liquid chromatographic method for the determination of the skeletal muscle relaxant baclofen in human plasma and urine is described Cation-exchange extraction, precolumn derivatization with o phthaldialdehyde, and on column concentration precede fluorimetric detection (excitation at 340 nm, emission at 460 nm) The precision of the assay was always better than 6% Recoveries of standards added to plasma and urine were 92% and 93%, respectively With a sample size of 0 5 ml, a detection limit of a few nanograms, and the possibility of analysing up to four samples per hour, this method is suitable for pharmacokinetic studies An example is presented

## INTRODUCTION

Baclofen, 4-amino-3-*p*-chlorophenylbutyric acid, is a skeletal muscle relaxant, which has been used in spastic disorders since its introduction for therapy in 1967 [1]. Several symposia have been dedicated to its pharmacological actions and clinical applications, but no conclusive evidence has yet been acquired for its mode of action [2—4]. Besides motor disorders other indications have also been proposed, such as schizophrenia [5—7], tardive dyskinesia [8, 9], and trigeminal neuralgia, but only the latter shows promising results [10, 11].

Baclofen is a *p*-chlorophenyl analogue of γ-aminobutyric acid (GABA), with the substituent rendering it a centre of asymmetry (Fig. 1). The discussion about its mode of action has been complicated by the different effects of the two enantiomers. The efficacy in spasticity has been attributed to (−)-baclofen, a substance with $GABA_B$-mimetic properties [12, 13] The commercially available drug (Lioresal®) is the racemic mixture.

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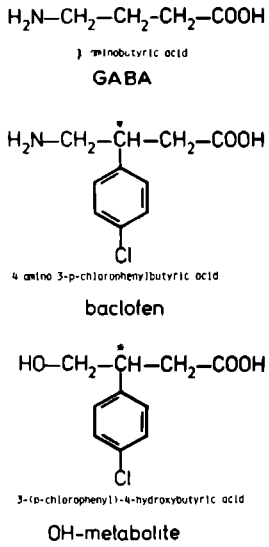


Fig. 1. Structural formulae of γ-aminobutyric acid (GABA), baclofen and its hydroxy metabolite.

Baclofen is metabolized to only a minor extent. Deamination yields 3-(*p*-chlorophenyl)-4-hydroxybutyric acid (Fig. 1), a metabolite which has been identified in the urine of rat, dog, and man. In man about 85% of a $^{14}$C-labelled oral dose was found to be excreted unchanged, primarily in the urine. Most of the remaining radioactivity was accounted for by the deaminated metabolite, which was inactive in animals [14, 15].

The development of a procedure to determine baclofen in body fluids encountered several analytical problems due to its amino acid structure. For the measurement of concentrations in the nanogram range, as needed for pharmacokinetic studies, it has to be separated from the endogenous amino acids. Degen and Riess [16] developed a gas—liquid chromatographic method with electron-capture detection, requiring lengthy derivatization reactions and resulting in only a 50% recovery. Gas chromatography in combination with mass spectrometry (GC—MS) has been described by Swahn et al. [17].

No high-performance liquid chromatographic (HPLC) method for the determination of baclofen has yet been published. One problem is that baclofen itself, like other amino acids, cannot be detected in low concentrations in biological material with ultraviolet spectrometry or fluorimetry. However, a considerable number of methods to assay endogenous amino acids, using reversed-phase HPLC in combination with fluorophore formation, have recently been published. Commonly used derivatization agents for pre- or postcolumn fluorescence detection of amino acids are Dns chloride, fluorescamine, and *o*-phthaldialdehyde (OPA) [18]. Derivatization with OPA is relatively simple and rapid. In the presence of alkylmercapto compounds highly fluorescent isoindoles are formed [19—21]. We developed an analytical assay for baclofen based upon precolumn derivatization with OPA. Separation

from the endogenous amino acids was achieved by cation-exchange extraction prior to the derivatization. High sensitivty was obtained with an on-column concentration and cleaning procedure which allows for injection of large volumes [22].

## MATERIALS AND METHODS

### *Reagents and chemicals*

Solutions were made in distilled water. All glassware was rinsed with distilled water prior to use. Chemicals were of analytical grade and were used without further purification. A stock solution of baclofen (a gift from Ciba-Geigy, Arnhem, The Netherlands) containing 100 mg/l was diluted with water, urine, or plasma to produce concentrations in the range 0.02—2 mg/l For the extraction procedure the following solvents and solutions were used hexane, methanol, saturated sodium chloride solution, citrate buffer pH 2.6 (0 1 *M* citric acid—0.2 *M* dibasic sodium phosphate, 89.1 10.9, v/v), and borate buffer pH 10.4 (0.1 *M* borax adjusted to pH 10.4 with sodium hydroxide) The derivatization reagent consisted of 250 mg of *o*-phthaldialdehyde dissolved in 1.5 ml of methanol, 23 ml of borate buffer pH 10.4 (0.4 *M* boric acid adjusted to pH 10.4 with potassium hydroxide), and 0.5 ml of thioglycolic acid The pH was adjusted to 10.4 after mixing [23]. Two mobile phases were used eluent A, 0.9% (w/v) sodium chloride solution, eluent B, methanol—tetrahydrofuran—phosphate buffer pH 8.5 (0.067 *M* dibasic sodium phosphate adjusted to pH 8.5 with monobasic potassium phosphate) (40.2·58, v/v/v).

### *Apparatus*

Extraction was performed with the Baker-10 extraction system (Baker Chemicals, Cat. No. 70180, Deventer, The Netherlands), fitted with 3-ml disposable extraction columns packed with aromatic sulphonic acid bonded to silica gel (Cat. No. 70903).

The chromatographic system consisted of a double-head solvent pump (Orlita, DHP-1515, Bakker, Zwijndrecht, The Netherlands), two sampling valves (Valco, Houston, TX, U.S.A.), and a sampling loop of 1.0 ml. Complete pulse quenching was achieved with a pulsation dampener (Orlita, PDM 3 350 M, Bakker) between pump and injection valve. The analytical column (25 cm × 4.6 mm I.D.) was packed with reversed-phase material Cp-Spher $C_8$, particle size 8 μm (Chrompack, Cat. No. 28502, Middelburg, The Netherlands) The concentration column (5 cm × 3.0 mm I.D.) was filled with LiChrosorb RP-8, 10 μm (Chrompack). A fluorescence detector (Perkin-Elmer, Model 3000, Delft, The Netherlands), equipped with a red sensitive photomultiplier and a doubly mirrored flow cuvette, was used. The detector was connected to a 10-mV recorder (Kipp & Zonen, BD 40, Delft, The Netherlands).

### *Extraction and derivatization*

The extraction column was conditioned with two column volumes of hexane, two column volumes of methanol, two column volumes of water, and three column volumes of saturated sodium chloride solution. To 0.5 ml of plasma (low concentrations 1.0 ml) or 0.5 ml of urine (high concentrations

were diluted prior to use) an equal volume of citrate buffer pH 2 6 was added This mixture was loaded onto the column After five subsequent column washings, four with water and the last with saturated sodium chloride solution, the sample was eluted with four 0 5 ml aliquots of borate buffer pH 10 4 To the collected eluent 0 4 ml of the derivatization reagent was added After mixing on a vortex mixer and subsequent centrifugation at 2000 *g*, a 1 0-ml sample was taken to be used for HPLC The time elapsed between the addition of the reagent until injection into the HPLC system was standardized at 150 sec

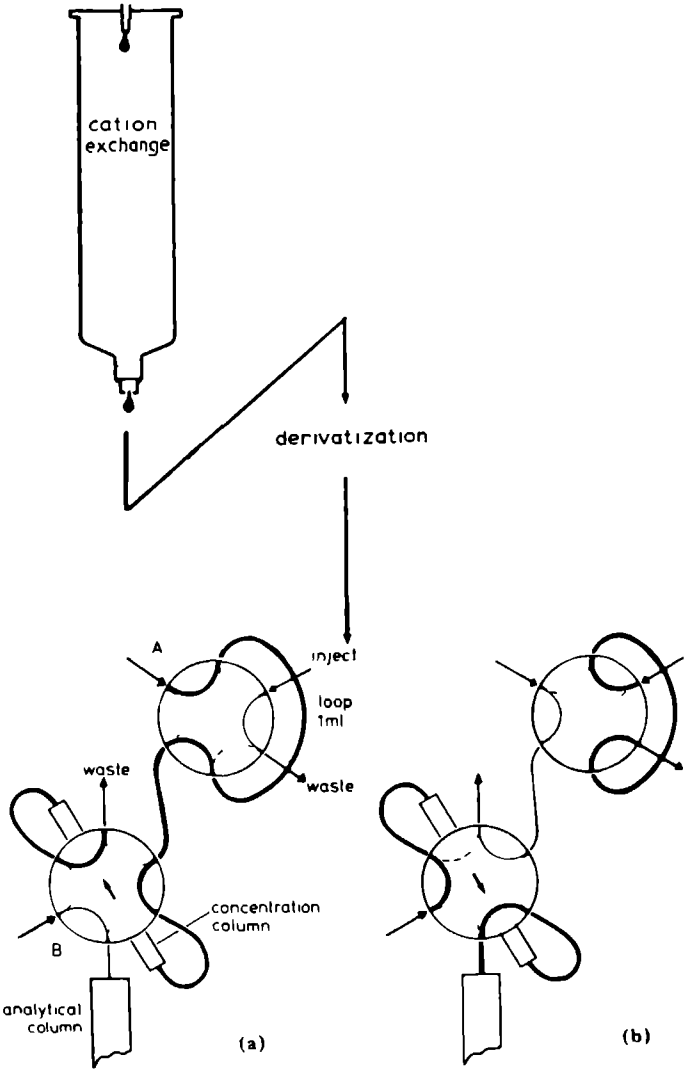


Fig 2 Schematic diagram of the assay of baclofen For further explanation, see text

*HPLC and detection*

The sample loop was filled as shown in Fig 2b By turning the upper valve, solvent A (flow-rate 1.5 ml/min, pressure approx. 5 MPa) transported the sample onto the concentration column (Fig. 2a) After 5 ml of solvent A had been used, the lower valve was turned and with 1.5 ml of solvent B (flow-rate 1 0 ml/min, pressure approx. 10 MPa) the concentrated sample could be flushed onto the analytical column (Fig. 2b) With an excitation wavelength of 340 nm and emission at 460 nm the fluorophore was detected quantitatively by measuring the peak height. The experiments were carried out at room temperature. Between samples the concentration column was flushed with several 1-ml methanol washings.

*Recovery*

The recoveries of the standards that had been added to water, plasma, and urine were measured in triplicate for three different concentrations in the range 0.10—2.1 mg/l and compared to a direct (i e. no extraction) assay in water.

*Experiment in a healthy volunteer*

A 35-year-old Caucasian woman (67 kg) was given a single oral dose of 20 mg baclofen (Lioresal®, two tablets of 10 mg each) 2 h after breakfast. Blood samples of 1—2 ml were drawn at predetermined intervals by fingertip puncture for a total period of 14 h. Samples from spontaneously voided urine were collected for 50 h. All blood specimens were collected in heparinized tubes. Plasma and urine were stored at −20° C until analysis.

## RESULTS AND DISCUSSION

Chromatograms of baclofen in plasma and urine are shown in Figs. 3 and 4. Blanks (Fig 3A and 4A) did not show interfering substances. In Fig 3B a plasma sample obtained from a patient treated with a daily oral dose of 45 mg is shown. The detection limit in plasma at a signal-to-noise ratio of 3 was approx. 1 5 ng (Fig. 3C). An example of baclofen measured in the urine of a volunteer is given in Fig. 4B. The detection limit in urine was approx. 5 ng (Fig. 4C). The capacity ratio ($k'$) was 3. Calibration curves showed good linearity between peak heights and concentrations ($r^2$ always > 0 99). The precision of the determination in water, plasma, and urine was measured for three different concentrations in the range 0.10—2.1 mg/l ($n$ = 4). Coefficients of variation were always less than 6% Recoveries in a similar concentration range were 97% for extraction from water, 92% and 93% for extraction from plasma and urine, respectively.

With the cation-exchange extraction procedure interfering endogenous amino acids could be effectively removed, owing to their low $pK_{a,1}$ values [24] as compared to baclofen ($pK_{a,1}$ = 3 87, $pK_{a,2}$ = 9 62) [15]. Thus, based upon the $pK_a$ values of baclofen and the pH needed for the subsequent derivatization reaction, the different buffers were chosen. When the conditioning with sodium chloride was omitted, baclofen was not reproducibly held on the column Prior to elution, another wash with sodium chloride was necessary for complete recovery. Derivatization of baclofen with OPA was as simple and rapid as for

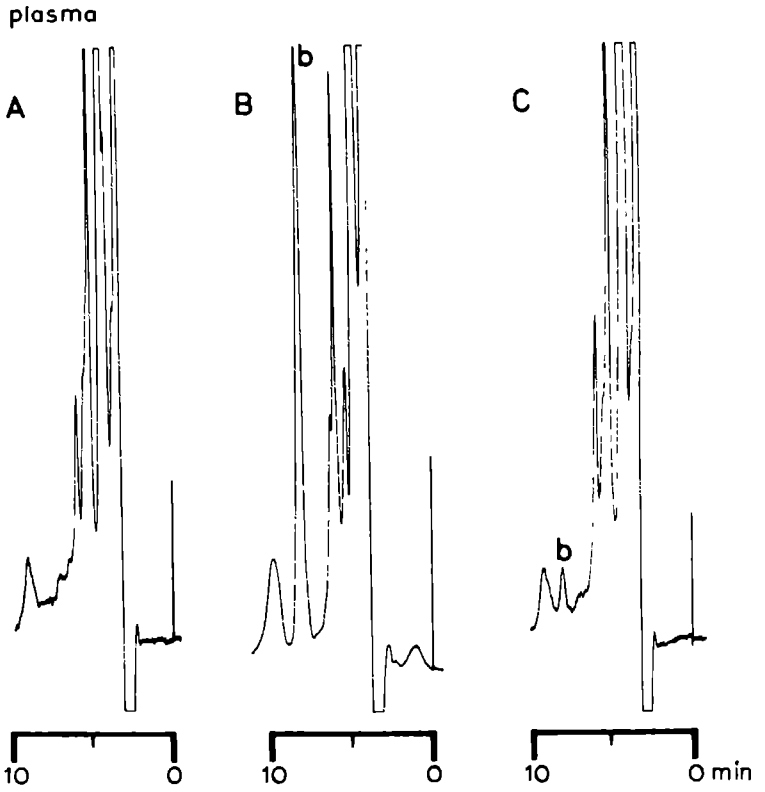


Fig. 3. Chromatograms of plasma samples: (A) plasma blank, (B) plasma of a patient on chronic oral therapy with 0.603 mg/l baclofen, (C) plasma spiked with 0.021 mg/l baclofen. b = baclofen.

endogenous amino acids. The structure of the proposed reaction product is given in Fig. 5. As the stability of the fluorescent derivatives of OPA with amino acids can vary with time [19—21], the baclofen fluorophore was always measured at a fixed time, in this case 150 sec after starting the reaction.

Although baclofen has been in clinical use for over fifteen years, hardly any pharmacokinetic data are available. Only a few studies have been published mentioning pharmacokinetic parameters, in volunteers [14, 15] and in patients [25, 26]. This seems to be mainly due to the lack of analytical procedures suitable for routine measurements.

To test the applicability of the presented HPLC method in pharmacokinetic studies a pilot experiment was done in a healthy volunteer. Fig. 6 shows the plasma concentration—time and renal excretion rate—time profiles of baclofen after a single oral dose of 20 mg. Some pharmacokinetic parameters are listed in Table I, calculated according to standard methods [27, 29]. The values of $t_{max}$ and of $C_{max}$ are similar to those reported by Swahn et al. [17] with the GC—MS method. After 50 h, 85% of the dose administered was recovered as unchanged drug in the urine. This is in agreement with data from experiments

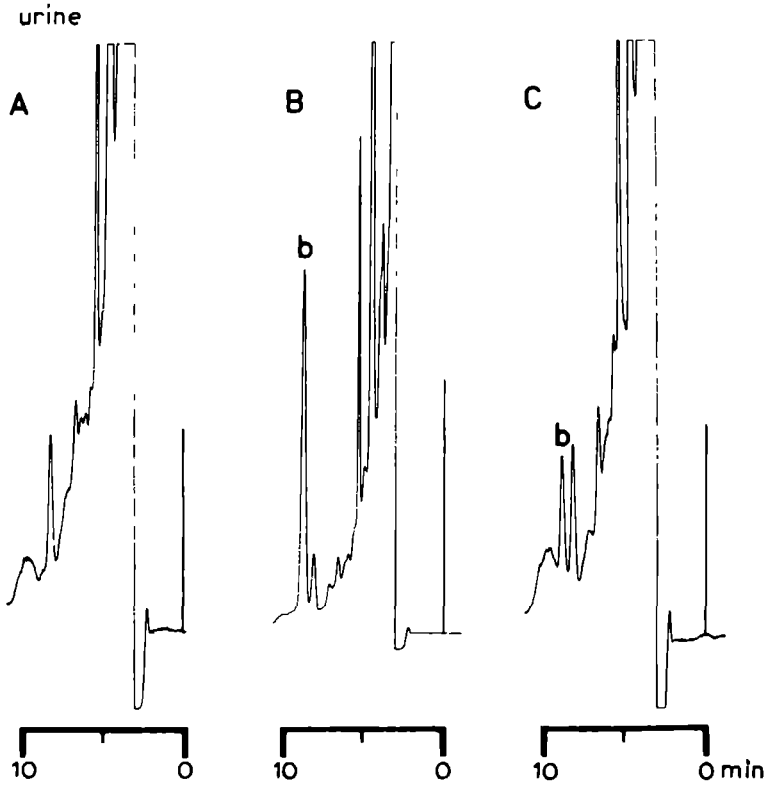


Fig. 4. Chromatograms of urine samples: (A) urine blank, (B) urine sample of a volunteer after a single oral dose of 2.51 mg/l baclofen, (C) urine spiked with 0.205 mg/l baclofen. b = baclofen.

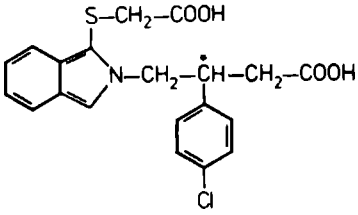


Fig. 5. Proposed reaction product of *o*-phthaldialdehyde and thioglycolic acid with baclofen.

with radioactively labelled baclofen [14, 15]. Clearance values have not been reported earlier. A total plasma clearance ($Cl$) of 0.16 l $h^{-1}$ $kg^{-1}$ was calculated. In this volunteer the renal clearance of baclofen ($Cl_R$) was equal to the creatinine clearance. Half-lives from plasma data have been reported to range from 2.5 to 6 h [14, 15]. After massive overdoses, however, half-lives of more than 30 h have been observed [30, 31]. We found a half-life of 5.4 h from terminal plasma data. When the combined plasma and urine data were fitted to a two-compartment open model using NONLIN [27], a higher terminal

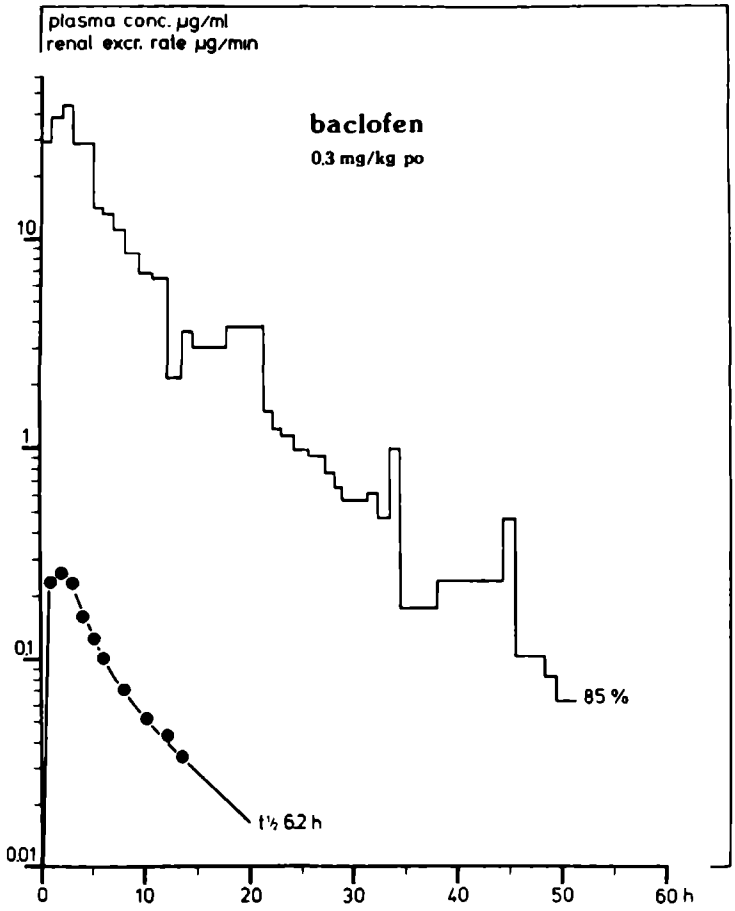


Fig. 6. Plasma concentration—time and renal excretion rate—time profiles of baclofen in a human volunteer following a single oral dose of 20 mg. % = percentage of the dose excreted unchanged in the urine.

TABLE I

SOME PHARMACOKINETIC PARAMETERS OF BACLOFEN IN MAN

Single oral dose of 20 mg ($n$ = 1). Abbreviations according to ref. 28, calculations according to refs. 27 and 29.

| | |
|---|---|
| $C_{max}$ | 0.24 mg/l |
| $t_{max}$ | 2.0 h |
| $t_{½z}$ | 6.2 h |
| $Cl^{*}$ | 0.16 l $h^{-1}$ $kg^{-1}$ |
| $Cl_R{}^{**}$ | 0.12 l $h^{-1}$ $kg^{-1}$ |
| $A_e(\infty)^{***}$ | 86% (of dose in urine) |

*Assumption $F$ = 1.
**Calculated from total plasma concentration data.
***Extrapolated to infinity.

half-life of 6 2 h was found In a preliminary study in eighteen patients on chronic oral therapy with daily baclofen doses ranging from 0 26 to 1 2 mg/kg, we measured plasma concentrations varying from 0 078 to 0 60 mg/l, with a mean total plasma clearance of 0 21 ± 0 11 l $h^{-1}$ $kg^{-1}$ Samples were drawn 3 h after the morning dose

With the described reversed phase HPLC method, only the parent drug is measured The hydroxymetabolite lacks the $NH_2$ group, which reacts with OPA Also no separation of the enantiomers is obtained These disadvantages also apply to the gas–liquid chromatographic methods From the results presented, the possibility of measuring up to four samples (plasma or urine) per hour, with a sample size of only 0 5 ml, and a detection limit of a few nanograms, it appears that baclofen can be measured with adequate sensitivity and selectivity for pharmacokinetic purposes

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Chapter 3

# RAPID SIMULTANEOUS DETERMINATION OF BACLOFEN AND ITS γ-HYDROXY METABOLITE IN URINE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET DETECTION

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*Biomedical Applications*
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## Note

# Rapid simultaneous determination of baclofen and its γ-hydroxy metabolite in urine by high-performance liquid chromatography with ultraviolet detection

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(First received February 16th, 1987, revised manuscript received April 3rd, 1987)

We have previously developed a method for the determination of the skeletal muscle relaxant baclofen (Fig 1) in plasma and urine by reversed-phase high-performance liquid chromatography (HPLC) with fluorimetric detection [1] This assay required cation-exchange extraction, pre-column derivatisation with *o*-phthaldialdehyde (OPA) and on-column concentration Although many samples have since been measured in this way, a simpler method was needed in order to speed up the analysis A rapid HPLC assay for baclofen in plasma was developed by Harrison et al [2]. This paper describes a rapid reversed-phase HPLC method with UV detection for the determination of baclofen in urine With this method it is also possible to measure the γ-hydroxy metabolite of baclofen (Fig 1) No separation of the enantiomers is obtained, however Recently, we published some preliminary results of an enantioselective assay [3]

## EXPERIMENTAL

### *Chemicals*

Baclofen and its γ-hydroxy metabolite (sodium salt) were kindly supplied by Ciba-Geigy (Basle, Switzerland) β-Glucuronidase was obtained from Sigma (St Louis, MO, U S A ) Hydrochloric acid, sodium hydroxide, sodium acetate, acetic acid, methanol and tetrahydrofuran were of analytical reagent grade (Merck, Darmstadt, F R G ) and were used without further purification

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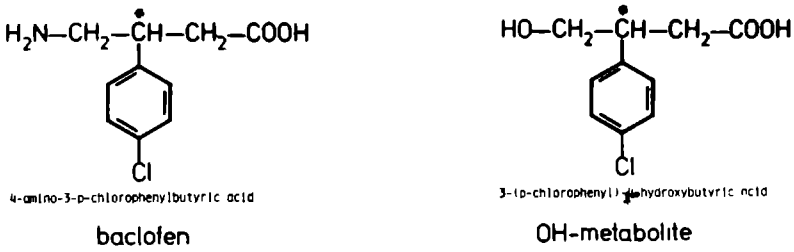


Fig. 1. Structures of baclofen and its $\gamma$-hydroxy metabolite.

*Apparatus*

A Model 9208 liquid chromatograph (Kipp Analytica, Delft, The Netherlands) with a variable-wavelength detector (Schoeffel SF 770 Spectroflow, GM 770 Monochromator; Kratos, Rotterdam, The Netherlands) was used. The analytical column (25 cm×4.6 mm I.D.) was packed with Cp-Spher $C_8$ reversed-phase material, particle size 8 $\mu$m (Chrompack, Middelburg, The Netherlands). A guard column (7.5 cm×2.1 mm I.D.) (Chrompack) was filled with pellicular reversed-phase material (Chrompack).

*Procedure*

Urine samples were diluted 1:3 with water and subsequently introduced into the HPLC system by means of a loop (50 $\mu$l). The mobile phase consisted of methanol–tetrahydrofuran–sodium acetate (0.02 *M*) (10:5:85, v/v/v). The flow-rate was 1 ml/min on average (pressure approximately 10 MPa). The substances were detected at 220 nm, and the peak heights were measured.

Deglucuronidation of urine samples was performed in three ways: enzymatically with $\beta$-glucuronidase and hydrolytic with hydrochloric acid or sodium hydroxide. In the enzymatic procedure, urine (0.3 ml) was incubated for 18 h at 37°C with 25 $\mu$l of $\beta$-glucuronidase (5000 U/ml) and 0.3 ml of a 0.2 *M* acetate buffer (pH 5.0). In the hydrolytic procedure, (a) urine (0.3 ml) was incubated for 2 h with 0.1 ml of 1 *M* hydrochloric acid at 95°C, then neutralised with 1 *M* sodium hydroxide, or (b) urine (0.3 ml) was incubated for 1 h with 0.1 ml of 1 *M* sodium hydroxide at 50°C, then neutralised with 1 *M* hydrochloric acid.

*Urine samples*

Baclofen was administered to dogs and human volunteers in different dosages. Urine was collected and stored at −20°C until analysis. More detailed results will be published elswhere.

*Dog experiment with the metabolite*

A female beagle dog of 11 kg body weight was anaesthetised with pentobarbitone sodium (30 mg/kg) and subsequently given 2 mg/kg $\gamma$-hydroxy metabolite as an intravenous (i.v.) infusion in 3 h. Urine samples were collected by means of a catheter for the first 7 h and subsequently spontaneously voided urine was used.

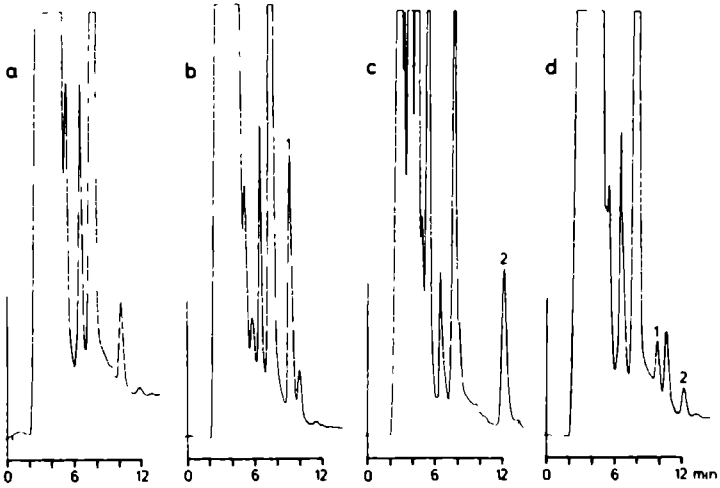


Fig. 2. Chromatograms of baclofen and its $\gamma$-hydroxy metabolite in urine. (a) Urine blank; (b) urine sample from a dog administered the $\gamma$-hydroxy metabolite i.v., containing 150 mg/l $\gamma$-hydroxy metabolite; (c) urine sample from a dog administered baclofen i.v. containing 84 mg/l baclofen; (d) urine spiked with 10.5 mg/l $\gamma$-hydroxy metabolite and 11.0 mg/l baclofen. Peaks: 1 = metabolite; 2 = baclofen.

## RESULTS AND DISCUSSION

Typical chromatograms of baclofen and its $\gamma$-hydroxy metabolite are shown in Fig. 2. A blank dog urine did not show interfering substances (Fig. 2a). However, depending on the nature of the samples, small day-to-day variations in the mobile phase and flow-rate may be necessary. Urine samples from a dog after i.v. administration of baclofen (Fig. 2c) and after i.v. administration of its $\gamma$-hydroxy metabolite (Fig. 2b) are shown. The detection limit in spiked urine was approximately 50 ng for both baclofen and its metabolite. The capacity ratios ($k'$) were 3.5 and 4.5 for the metabolite and baclofen, respectively. For both substances calibration graphs of blank urine, spiked with 4.0–100 mg/l and measured after diluting 1:3 with water, showed good linearity between peak height and concentration ($r^2 > 0.99$). The precision of the determinations in water and in urine was measured for three different concentrations within the same range ($n = 4$). The coefficients of variation were always less than 4% for both baclofen and its metabolite. Comparison of the direct UV method with the *o*-phthaldialdehyde (OPA) fluorimetric determination for baclofen showed a good correlation (Fig. 3). The correlation coefficient ($r^2$) based on fourteen samples was 0.99.

According to Faigle et al. [4], following the administration of $^{14}$C-labelled baclofen 80–90% of the $^{14}$C dose was excreted in the urine in the dog and in man. In man 90% of the total radioactivity was accounted for by the unchanged drug and 7% by 3-(*p*-chlorophenyl)-4-hydroxybutyric acid, the $\gamma$-hydroxy metabolite (Fig. 1). In the dog these values were 63 and 8%, respectively. In our experiments with unlabelled baclofen the recovery of the unchanged drug in the urine was

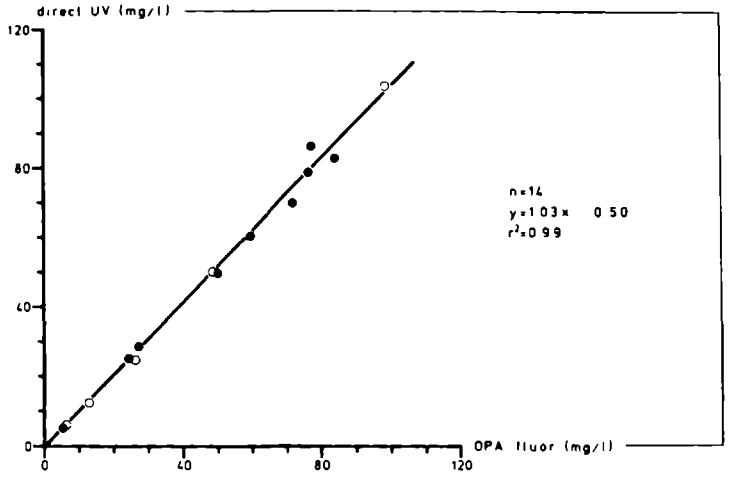


Fig 3 Correlation of the direct UV method with the OPA fluorimetric method for the determination of baclofen in urine o, Spiked samples, ●, samples from dog urine following i v administration of baclofen

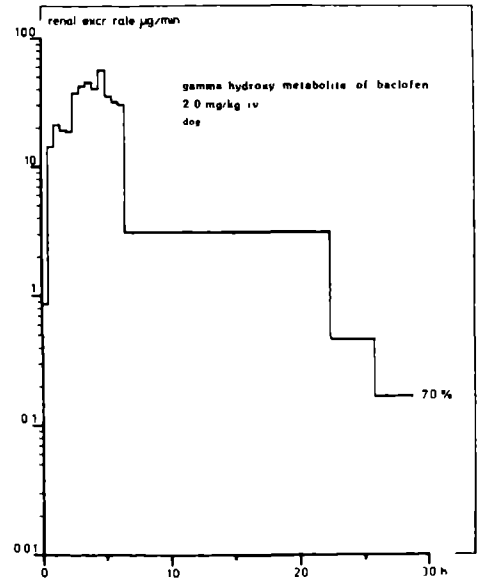


Fig 4 Renal excretion rate-time profile of the γ-hydroxy metabolite of baclofen, administered as the parent compound to a dog Dose 2 mg/kg as i v infusion % = percentage of the dose excreted unchanged in the urine

variable both in man and in the dog, with values ranging from 40 to 90% of the dose administered

With the direct UV method the urine samples could also be screened for the presence of the γ-hydroxy metabolite The earlier described OPA fluorimetric method could not be used as the metabolite lacks an $NH_2$ group, which is needed for derivatisation As theoretically the metabolite (and also baclofen itself) could have been present as the corresponding glucuronide, the samples were analysed prior to and after enzymatic and hydrolytic deglucuronidation

In urine samples from both dogs and human volunteers following i v administration of baclofen, however, no metabolite was found Given the detection limit of the method, this means that in 48 h, depending on the urinary flow-rate, 2–10% of a dose at the most could have been present as the γ-hydroxy metabolite, without having been detected No glucuronides were found Because of the urinary pH values (5–9) it is unlikely that the γ-hydroxy metabolite in the urine samples analysed would have been present as the corresponding lactone, which is not detected with this method.

When the γ-hydroxy metabolite was administered to a dog as the parent compound, 70% was recovered unchanged in the urine in 30 h (Fig 4) This means that the described assay is suitable for the measurement of the γ-hydroxy compound if present in sufficiently high concentrations Apparently, the incomplete mass balance of baclofen is mainly due to other causes

With this simple reversed-phase HPLC method, baclofen can be measured in urine samples For low concentrations (less than 4 mg/l), the more lengthy OPA fluorimetric method should be used. The γ-hydroxy metabolite can also be measured in urine by the direct UV method if present at concentrations higher than 4 mg/l For separation of the enantiomers other methods are required.

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Chapter 4

# ENANTIOSELECTIVE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF BACLOFEN AFTER DERIVATIZATION WITH A CHIRAL ADDUCT OF o-PHTHALDIALDEHYDE

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## Note

# Enantioselective high-performance liquid chromatographic determination of baclofen after derivatization with a chiral adduct of *o*-phthaldialdehyde

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(First received September 1st, 1986; revised manuscript received November 5th, 1986)

Baclofen (4-amino-3-*p*-chlorophenylbutyric acid), a skeletal muscle relaxant used in the treatment of spastic disorders, is administered clinically as a racemic mixture. In animals, *R*(−)-baclofen (Fig. 1) is the more potent isomer responsible for the $GABA_B$-mimetic action [1–5]. *S*(+)-Baclofen interferes with the binding of *R*(−)-baclofen, and has been proposed to act as an antagonist at the $GABA_B$-receptors [6,7]. Pharmacokinetic properties of the separate enantiomers have not yet been described. To be able to examine the optical purity of baclofen preparations, a reliable analytical method is required, for pharmacokinetic purposes to be extended to measurements in biological fluids.

Stereoselective analysis of a mixture of enantiomers is difficult. In high-performance liquid chromatography (HPLC), separation is possible through the use of chiral eluents or chiral stationary phases or through derivatization with chiral reagents [8,9]. For optical resolution of the baclofen enantiomers for preparative purposes, Weatherby et al. [10] used a chiral mobile phase. This paper describes the first results of a modification of our recently developed assay for racemic baclofen, based on derivatization with *o*-phthaldialdehyde (OPA) in the presence of thiol compounds [11]. The optically inactive thioglycolic acid was substituted for N-acetyl-L-cysteine (NAC), a method originally developed for the optical resolution of amino acids [12,13].

## EXPERIMENTAL

### *Chemicals*

*o*-Phthaldialdehyde and N-acetyl-L-cysteine were obtained from Merck (Darmstadt, F.R.G.) and *R,S*-, *R*(−)- and *S*(+)-baclofen were a gift from Ciba-

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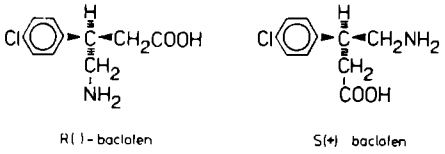


Fig 1 Enantiomers of baclofen

Geigy (Basle, Switzerland). Solutions were prepared in distilled water. All glassware was rinsed with distilled water prior to use. All other chemicals were of analytical-reagent grade and were used without further purification

*Chromatographic system*

The chromatographic system was similar to that described previously [11] a reversed-phase system with on-column concentration and fluorimetric detection (excitation at 340 nm, emission at 460 nm) The only differences were the analytical column (Chrompack Cp-Spher $C_8$, 25 cm, Cat No. 28310) and solvent B [43–50% (v/v) methanol, 2 5% (v/v) tetrahydrofuran and 54 5–47 5% (v/v) phosphate buffer (pH 8 5)]

*Pre-column derivatization procedure*

For the preparation of the OPA–NAC reagent, boric acid (0 62 g) and NAC (1 12 g) were dissolved in 20 ml of water and adjusted to pH 10 4 with sodium hydroxide OPA (250 mg) dissolved in 3 ml of methanol was added and diluted with water to a final volume of 25 ml This solution was kept at 4 °C and prepared freshly every week

To 0 9 ml of a sample solution of baclofen containing 5–500 ng was added 0 2 ml of OPA–NAC reagent and the mixture was allowed to stand for 25 min at 80 °C The solution was then cooled in ice for 1 min and 25 $\mu$l of 60 m$M$ sodium acetate solution were added After mixing, 1 0 ml was taken for direct injection into the HPLC system

## RESULTS AND DISCUSSION

Fig 2 shows chromatograms of the OPA–NAC derivatives of $R(-)$- and $S(+)$-baclofen A blank run did not show interfering substances (Fig 2a) The detection limit is approximately 2 ng (Fig 2c) The fluorescent derivatives of $R(-)$- and $S(+)$-baclofen have capacity ratios of 2 7 and 3 1, respectively, and a resolution of 1 0 The calibration graphs for both derivatives showed good linearity between peak heights and concentrations in the range 20–200 ng per injection ($r^2 > 0\ 99$) The precision of the determination was measured for three different concentrations of each enantiomer (35, 90 and 205 ng/ml) ($n = 4$) and the coefficients of variation were less than 4%

Derivatization of baclofen with the OPA–NAC reagent was not as simple and rapid as described for a series of amino acids [12,13] If the same reaction conditions were chosen as for the amino acids, following injection of racemic baclofen

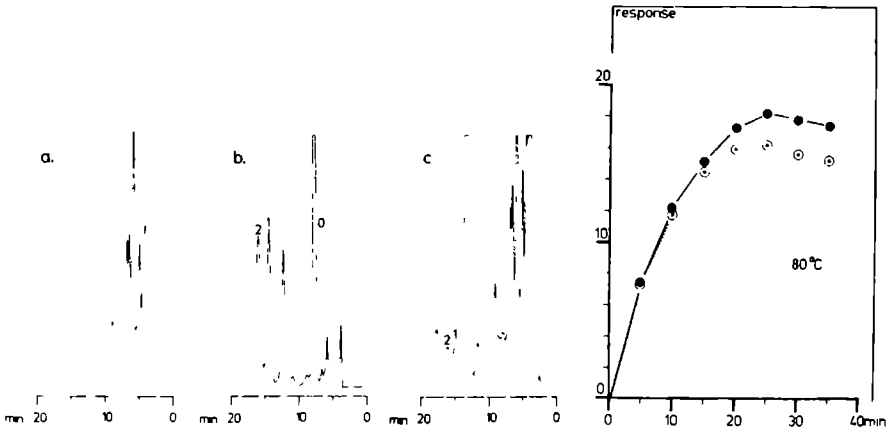


Fig. 2. Chromatograms of OPA-NAC derivatives of $R(-)$- and $S(+)$-baclofen. The injected sample contained (a) 0 ng (blank), (b) 410 ng and (c) 4.0 ng of $R,S$-baclofen. Peaks: 1 = $R(-)$-baclofen; 2 = $S(+)$-baclofen; 0 = unresolved reaction product.

Fig. 3. Example of the OPA-NAC derivatization with baclofen as a function of reaction time at 80°C. The injected sample contained 40 ng of $R,S$-baclofen; the fluorescence was measured as peak height. ●, $R(-)$-Baclofen; ○, $S(+)$-baclofen.

three peaks were observed on the chromatogram that were not present in the blank sample. Under these conditions (3 min at room temperature), peak 0 (Fig. 2b) had the highest fluorescent response, its stability being comparable to that of the previously described OPA–NAC primary amine derivatives, i.e., a decomposition half-life of about 3 h at room temperature [12]. The other two components on the chromatogram, corresponding to derivatives of $R$-(−)- and $S$-(+)-baclofen (Fig. 2, peaks 1 and 2) gave a maximum response beyond a reaction time of 7 h at room temperature. At 80°C this time could be reduced to 25 min (Fig. 3), with reproducible results.

The reaction mechanism is not clearly understood. An explanation could be the formation of 1,3-dithio-substituted isoindoles, due to autoxidation [14]. As all OPA-derived isoindoles have limited stability depending on parameters not yet fully known, the reaction time and temperature should be kept constant [15,16].

Additional studies in biological fluids are currently being carried out.

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Chapter 5

# SIMULTANEOUS DETERMINATION OF DANTROLENE AND ITS METABOLITES, 5-HYDROXYDANTROLENE AND NITRO-REDUCED ACETYLATED DANTROLENE (F 490), IN PLASMA AND URINE OF MAN AND DOG BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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*Biomedical Applications*
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CHROMBIO 1336

# SIMULTANEOUS DETERMINATION OF DANTROLENE AND ITS METABOLITES, 5-HYDROXYDANTROLENE AND NITRO-REDUCED ACETYLATED DANTROLENE (F 490), IN PLASMA AND URINE OF MAN AND DOG BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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## SUMMARY

A reversed phase high-performance liquid chromatographic method is described for the simultaneous determination of the skeletal muscle relaxant dantrolene and its metabolites, 5-hydroxydantrolene and nitro-reduced acetylated dantrolene (F 490), in plasma and urine of man and dog The substances are detected spectrophotometrically at 375 nm The detection limits are 0 02 mg/l A preliminary extraction step into a chloroform—butanol mixture is required for the plasma samples The method is suitable for pharmacokinetic studies of dantrolene

## INTRODUCTION

Dantrolene sodium, 1-{[5-(*p*-nitrophenyl)-furfurylidene]-amino}-imidazolidine-2,4-dione sodium salt hydrate, first reported by Snyder et al. [1], is used as a skeletal muscle relaxant which appears to act by blocking muscle contraction beyond the neuromuscular junction [2—5] It is used for the symptomatic relief of clinical spasticity resulting from serious disorders such as cerebral palsy, stroke, spinal cord injury, and multiple sclerosis [6—13]. More recently, it has also been recommended for the prevention and treatment of malignant hyperthermia, a syndrome recognized as one of the causes of anaesthesia-related deaths [14—16].

Metabolism of dantrolene (see Fig 1) has been shown to proceed through both reductive and non-reductive pathways [17]. The nitro group of dantrolene is reduced to the corresponding amine, and in man and some animals subsequently acetylated to yield nitro-reduced acetylated dantrolene (F 490).

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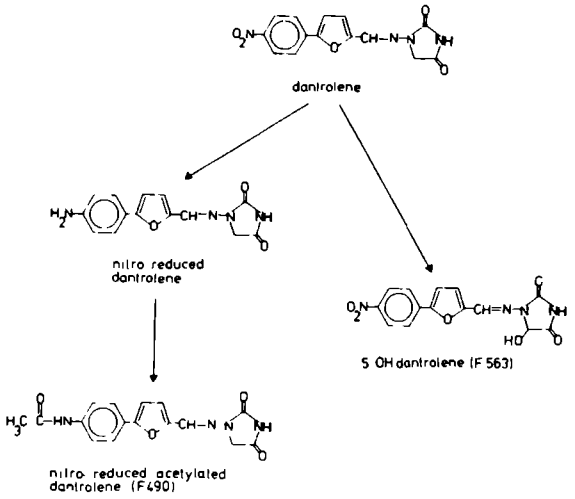


**Fig 1 Proposed metabolic scheme of dantrolene [18]**

Of these reduced metabolites, which do not possess muscle relaxant properties [18], only F 490 has been detected in human blood [17,19] and urine [17,20]. Oxidation of dantrolene results in 5-hydroxydantrolene [21], a metabolite with muscle relaxant effects, the activity is less than dantrolene on an equimolar basis [18]. This 5-hydroxy metabolite has been identified in human blood [17,19,20,22,23] and urine [17,20].

Several analytical methods have been developed for the determination of dantrolene in blood and urine, including spectrophotofluorimetry [24,25], differential pulse polarography [17], high-performance liquid chromatography (HPLC) [26,27], and a qualitative colorimetric procedure [28]. The fluorimetric and polarographic methods require complicated differential analytical techniques to measure the drug in the presence of its metabolites. Meyler et al [22], who used the fluorimetric procedure for plasma concentration measurements in volunteers and patients, encountered a discrepancy in the method which was not further investigated. They found that the total fluorescence of an extract from plasma containing dantrolene and 5-hydroxydantrolene (direct method) was considerably less than the fluorescence measured after the extract had passed through a Sephadex column and the two fractions containing 5-hydroxydantrolene and dantrolene, respectively, had been combined (indirect method).

The first reported HPLC procedure for dantrolene has the disadvantage of using a non-aqueous mobile phase [19,26]. In a short communication Hackett and Dusci [27] reported a reversed-phase HPLC procedure with, however, limited sensitivity and selectivity. Only dantrolene itself was measured, in concentrations down to 0.25 mg/l. To investigate the pharmacokinetics of dantrolene in volunteers and patients a new reversed-phase HPLC procedure was developed which allows the measurement of the drug and its metabolites at levels as low as 0.02 mg/l.

*Reagents and chemicals*

All chemicals were of analytical grade. Three separate standard solutions were prepared in N,N-dimethylformamide containing 0.5, 0.5 and 0.3 mg/ml dantrolene sodium, 5-hydroxydantrolene, and nitro-reduced acetylated dantrolene (F 490), respectively, (a gift from Eaton Laboratories, Norwich, NY, U.S.A.). Ultrasonic treatment was used to increase the rate of dissolution of F 490. The extraction fluid was chloroform–1-butanol (95:5, v/v). Phosphate buffer (pH 6.8) was 50% (v/v) of dibasic sodium phosphate·$2H_2O$ solution (11.88 g/l) and 50% (v/v) of monobasic potassium phosphate solution (9.08 g/l). The mobile phase was acetonitrile—phosphate buffer (pH 6.8) (33.3:66.6, v/v).

*Apparatus and chromatographic conditions*

A liquid chromatograph (Kipp Analytica No. 9208) with a variable-wavelength detector (Schoeffel Instruments; SF 770 Spectroflow, GM 770 Monochromator), and a column packed with CP-Spher $C_8$ (Chrompack, Middelburg, The Netherlands; Cat. No. 28502, particle size 8 $\mu$m, 25 cm × 4.6 mm I.D.) was used. The flow-rate was 1.5 ml/min (pressure approx. 5 MPa). Samples were introduced by means of a 50-$\mu$l loop. The substances were detected at 375 nm, and the peak heights were measured.

*Plasma*

*Calibration curve* A mixture of the standard solutions was diluted with blank plasma (concentrations 0 02—4 mg/l). To 1 0 ml of each solution were added 0 5 g of ammonium sulfate and 4.0 ml of the extraction fluid. The solutions were shaken for 10 min in a rotary mixer (Cenco Instruments, Cat. No. 23426). After centrifugation for 10 min at 2000 *g* (Heraeus Christ, type UJ1S), an aliquot of the lower layer was collected and evaporated to dryness in a hot (50°C) water bath under nitrogen. The residue was mixed with 1.0 ml of the mobile phase on a vortex mixer and injected onto the column. A control with N,N-dimethylformamide in blank plasma was treated in the same manner.

*Samples.* To 1.0 ml of plasma were added 0.5 g of ammonium sulfate and 4.0 ml of the extraction fluid; this mixture was further treated as described for the calibration curve.

*Urine*

*Calibration curve.* A mixture of the standard solutions was diluted with blank urine and diluted further 1 in 10 with the mobile phase to final concentrations of 0.02—4 mg/l. These solutions were directly injected onto the column. A control with N,N-dimethylformamide in blank urine was treated in the same manner.

*Samples* Urine samples of 100 $\mu$l were diluted 1 in 10 with the mobile phase and directly injected.

*Dog experiment*

A male beagle dog of 12 kg body weight was anaesthetized with pento-

barbitone and subsequently given 12 mg of dantrolene sodium (dantrium) intravenously. Blood samples were collected at scheduled intervals Urine samples were collected by means of a catheter for the first 7 h, thereafter spontaneously voided urine was used.

*Recovery*

Solutions in urine, prepared as described under the calibration curve for urine, and extracts from water and plasma, using the procedure as described under *Calibration curve* for plasma, were compared to a direct assay of standards in the mobile phase. The recoveries were determined for three different concentrations.

*Stability*

The standard solutions, which were kept protected from light at 4°C, were periodically measured spectrophotometrically (Beckman spectrophotometer, Model 3600), and by the described HPLC procedure.

## RESULTS

A chromatogram of a plasma sample obtained from a patient treated with a daily oral dose of 5 mg/kg body weight dantrolene sodium is given in Fig. 2.

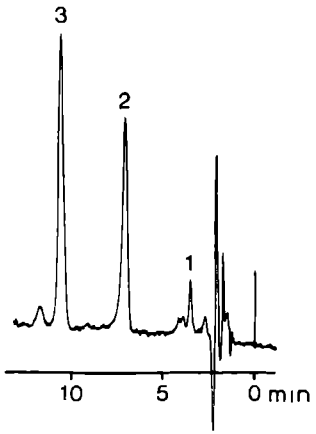


Fig 2 Liquid chromatogram of patient plasma containing 0.705 mg/l dantrolene (3), 0.624 mg/l 5-hydroxydantrolene (2), and 0 055 mg/l F 490 (1)

Calibration curves in plasma and urine showed good linearity between peak heights and concentrations from 0.02 to 4.0 mg/l ($r^2$ = 0.999 for all substances). Chromatograms of blanks did not show any interfering substances at the detection wavelength of 375 nm. Retention times and capacity ratios are given in Table I.

The detection limit of dantrolene and its metabolites was 1 ng, defined as three times the noise level. The precision of the determination was measured for two different concentrations; the coefficients of variation are given in

TABLE I

RETENTION TIMES OF DANTROLENE AND ITS METABOLITES

For chromatographic conditions, see text

| Substance | Retention time (min) | Capacity ratio ($k'$) |
|---|---|---|
| Dantrolene | 10 9 | 3 5 |
| 5 Hydroxydantrolene | 7 1 | 2 0 |
| F 490 | 4 1 | 0 7 |

TABLE II

COEFFICIENTS OF VARIATION

For description of analytical procedure, see text

| Substance | Plasma | | | Urine | | |
|---|---|---|---|---|---|---|
| | Concentration (mg/l) | Coefficient of variation (%) | $n$* | Concentration (mg/l) | Coefficient of variation (%) | $n$* |
| Dantrolene | 0 051 | 3 5 | 6 | 0 043 | 7 0 | 6 |
| | 1 63 | 3 9 | 5 | 1 30 | 5 7 | 6 |
| 5 Hydroxydantrolene | 0 066 | 3 1 | 6 | 0 055 | 6 0 | 6 |
| | 2 13 | 4 3 | 5 | 1 68 | 6 3 | 6 |
| F 490 | 0 039 | 6 1 | 4 | 0 031 | 5 8 | 6 |
| | 1 24 | 3 9 | 5 | 1 07 | 6 3 | 6 |

*$n$ = number of determinations

TABLE III

RECOVERIES OF DANTROLENE AND ITS METABOLITES

For description of analytical procedure, see text

| Substance | Recovery* (%) | | |
|---|---|---|---|
| | Water (extracted) | Plasma (extracted) | Urine |
| Dantrolene | 97 ± 4 | 99 ± 8 | 103 ± 7 |
| 5 Hydroxydantrolene | 100 ± 3 | 86 ± 2 | 99 ± 1 |
| F 490 | 101 ± 4 | 89 ± 4 | 101 ± 4 |

*Means and standard deviations of three different concentrations ($n$ = 3) dantrolene 3 25, 0 406, and 0 051 mg/l, 5 hydroxydantrolene 4 20, 0 525, and 0 066 mg/l, F 490 2 68, 0 335, and 0 042 mg/l

Table II. The results of the recovery experiments are mentioned in Table III.

The standard solutions of all three substances did not show any deterioration for at least three months when kept at 4°C and when protected from light. Upon standing in light, however, F 490 deteriorates extremely quickly. On the chromatogram two peaks develop (Fig. 3). After 4 h at room temperature only about 50—60% of the peak heights relative to a freshly prepared solution could be measured (Fig. 4).

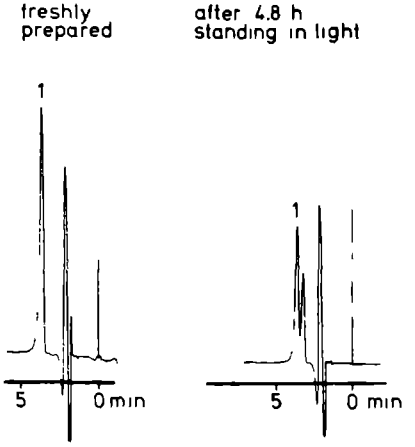


Fig. 3. Liquid chromatograms of a solution containing F 490 (1), showing instability upon standing in light.

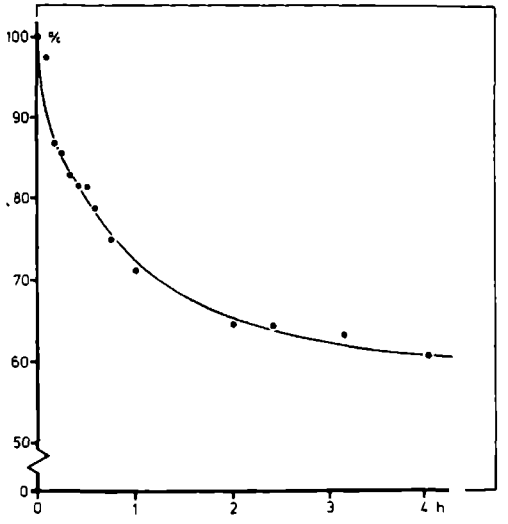


Fig. 4. Degradation of a solution containing F 490 upon standing in light.

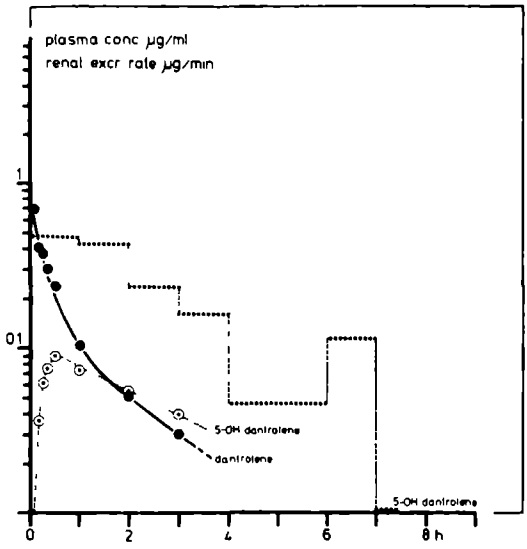


Fig. 5. Plasma concentration and renal excretion rate profiles of dantrolene and 5-hydroxydantrolene in a dog after intravenous administration of 1 mg/kg dantrolene sodium.

Fig. 5 shows the plasma concentration and renal excretion rate profiles of dantrolene and 5-hydroxydantrolene in a dog after intravenous administration of 1 mg/kg body weight dantrolene sodium. No dantrolene is excreted unchanged in dog urine.

## DISCUSSION

The very low solubility of dantrolene and its metabolites in many solvents, including water, and the degradation by light, are complicating factors in the analysis. Dantrolene sodium is slightly soluble in water (15 mg/l), but it hydrolyzes quickly. The extremely insoluble (less than 1 mg/l) free acid dantrolene precipitates. The water solubilities of 5-hydroxydantrolene and F 490 are less than 10 mg/l (Data Sheet, Eaton Laboratories). Protection from light during the whole procedure appears to be important, especially for F 490, which is very unstable upon standing in light (see Figs. 3 and 4). N,N-dimethylformamide appeared to be a suitable solvent in preparing standard solutions. The solutions were stable for at least three months when kept protected from light at 4°C. No interference from this solvent was observed in the chromatographic assay.

Extraction of 1 ml of plasma with 4 ml of the chloroform—1 butanol (95:5) mixture yielded good recoveries. For the recovery of dantrolene from plasma (see Table III) a higher value than reported by Saxena et al. [26] was found; they used 80 ml of a chloroform—butanol (70:30) mixture.

On the chromatogram, the peaks of parent drug and metabolites were well resolved (see Fig. 2). With the reversed-phase method of Hackett and Dusci

[27] a relatively poor resolution between dantrolene and 5-hydroxydantrolene was obtained

Slightly different chromatographic conditions may be needed for measurements in urine samples because of interference by endogenous substances with low retention times

At present only a few pharmacokinetic studies of dantrolene have been published Varying values for plasma concentrations and drug metabolite ratios have been reported Vallner et al [19] found dantrolene plasma concentrations between 0 03 and 0 2 mg/l, with slightly higher values for the metab olites in patients on chronic oral therapy, with daily dosages ranging from 50 to 200 mg However, Lietman et al [20] and Meyler [23] found plasma dantrolene concentrations of between 1 and 2 mg/l in patients on daily dosages of 4—12 mg/kg and 200—400 mg, respectively, the concentration of the 5-hydroxymetabolite appeared to be only 30—50% that of the parent com pound In a preliminary survey of patients on low dosages (0 7—5 mg/kg) we found large inter-individual differences in plasma concentrations and drug metabolite ratios, with plasma concentrations of 0 2—2 0 mg/l, 0 1—1 0 mg/l, and about 0 02 mg/l for dantrolene, 5-hydroxydantrolene, and F 490, respec tively In the dog experiment dantrolene and 5-hydroxydantrolene could be detected in plasma up to 3 h after the intravenous dose The amount of 5-hydroxydantrolene excreted in the urine accounted for less than 1% of the dose No dantrolene or F 490 could be detected in the urine

The reported differences in the concentrations of the drug and of the metab olites have stimulated the investigation of the clinical pharmacokinetics of dantrolene. The described method provides adequate sensitivity and selectivity to make it applicable to pharmacokinetic studies

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Chapter 6

# DETERMINATION OF A DANTROLENE METABOLITE, 5-(p-NITROPHENYL)-2-FUROIC ACID, IN PLASMA AND URINE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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*Biomedical Applications*
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## Note

# Determination of a dantrolene metabolite, 5-(*p*-nitrophenyl)-2-furoic acid, in plasma and urine by high-performance liquid chromatography

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The direct-acting muscle relaxant dantrolene (Fig. 1) is used in patients with spasticity and also in humans and animals, including dogs at risk of malignant hyperthermia [1,2]. Although its efficacy is well established, knowledge concerning the fate of this drug in the body is limited owing to the complicated and extensive metabolism, which is also the case for the structurally related nitrofurans [3]. Known metabolic pathways for dantrolene are hydroxylation of the hydantoin ring and reduction of the nitro group, which may then be acetylated [1]. Many methods have been developed for the quantification of dantrolene and its oxidized and reduced metabolites in plasma and urine, the latest by Lalande et al. [4], but when these are applied to pharmacokinetic studies, the major part of the mass balance is missing. A pilot study in the dog showed that less than 1% of the intravenously administered dose was excreted in the urine [5] and renal recovery in humans amounted to only 15% [6]. Other pathways of elimination must exist to explain this incomplete recovery.

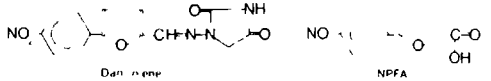


Fig. 1. Structures of dantrolene and its metabolite 5-(*p*-nitrophenyl)-2-furoic acid (NPFA).

0378-4347/90/$03.50 

Cleavage of the azomethine (-CH=N-) linkage is one pathway in need of further investigation. Analogously to nitrofurans [3,7], in vitro acidic hydrolysis at body temperature has resulted in aldehyde formation [8], and the corresponding acid, 5-(*p*-nitrophenyl)-2-furoic acid (NPFA) (Fig. 1), has been identified in vivo (Norwich Eaton Pharmaceuticals, product information). This paper describes a modification of our previously developed assay for dantrolene [5], which allows quantification of NPFA in plasma and urine of humans and dogs.

## EXPERIMENTAL

### *Reagents and chemicals*

All chemicals were of analytical grade. Norwich Eaton Pharmaceuticals (Norwich, NY, U.S.A.) kindly supplied NPFA. A stock solution (0.5 mg/ml) was prepared in N,N-dimethylformamide and kept in the dark at 4°C. Phosphate buffer (pH 6.8) was 50% (v/v) of dibasic sodium phosphate dihydrate solution (11.88 g/l) and 50% (v/v) of monobasic potassium phosphate solution (9.08 g/l).

### *Apparatus and chromatographic conditions*

The HPLC system was similar to that previously described [5]. The mobile phase, acetonitrile–phosphate buffer (25:75, v/v for plasma, 20:80, v/v for urine), was used at room temperature at a flow-rate of 1.5 ml/min with a CP-Spher$C_8$ column (25 cm×4.6 mm I.D., particle size 8 μm) (Chrompack, Middelburg, The Netherlands). NPFA was detected at 354 nm.

### *Procedure*

*Plasma.* To standard or unknown plasma (200 μl) in a small glass centrifuge tube, 50 μl of 2 *M* hydrochloric acid and 1.4 ml of the extraction mixture, chloroform–1-butanol (95:5, v/v) were added. After vortex-mixing for 60 s, the tubes were centrifuged at 2000 *g* for 5 min. An aliquot of the lower layer (1.0 ml) was then collected and evaporated to dryness in a water-bath (37°C) under nitrogen. The residue was reconstituted with 250 μl of the mobile phase and subsequently injected into the liquid chromatograph (50-μl loop).

*Urine.* Standard or unknown urine (200 μl) was mixed with 200 μl of the mobile phase and directly injected (50 μl).

Calibration curves were constructed by plotting the concentrations against the peak heights. The calibration range was based on the expected concentrations and established in the range 0.1–10 mg/l.

### *Recovery experiments*

Samples containing four concentrations of the standard compound in plasma in the range 0.1–10 mg/l were prepared in duplicate and treated as described

above. The percentage recovery was determined by comparing the heights of the peaks obtained with the heights obtained from standards that were diluted with mobile phase to identical concentrations and directly injected.

*Samples*

Dantrolene was administered to dogs and human volunteers in different dosages. Dogs also received 5-hydroxydantrolene. Plasma and urine were collected and stored in the dark at −20 °C until analysis. More details will be published elsewhere.

*Dog experiment with the metabolite*

A female Beagle dog with a body weight of 14 kg was anaesthetized with pentobarbitone and subsequently given 13 mg of NPFA by constant-rate intravenous infusion for 45 min. Serial 2-ml blood samples were collected in heparinized tubes at scheduled intervals. Urine samples were collected by means of a catheter for the first 7 h, and subsequently spontaneously voided specimens were used. Urinary pH was measured in each sample.

## RESULTS AND DISCUSSION

Typical chromatograms of NPFA in dog plasma and urine are shown in Figs. 2 and 3. Neither the chromatograms of the drug-free specimens for the dog (A) nor those for the human volunteers contained any interfering substances. The concentration of NPFA in the spiked samples (B) was 0.49 mg/l (plasma) and 0.57 mg/l (urine). After intravenous administration of 5-hydroxydantrolene (3.9 mg/kg) the concentrations of NPFA in the samples shown (C) were 0.86 mg/l (plasma) and 0.84 mg/l (urine). The detection limit of NPFA was ca. 1 ng, defined as three times the noise level. The retention times ($t_R$) were 3.4 and 7.4 min for plasma and urine, respectively. Calibration graphs showed good linearity in the ranges mentioned ($r^2$ always greater than 0.99). The precision

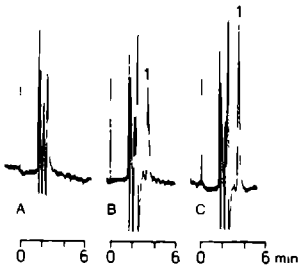


Fig. 2 Chromatograms of (A) drug-free dog plasma, (B) dog plasma spiked with 0.49 mg/l NPFA, (C) dog plasma sample taken after intravenous administration of 5-hydroxydantrolene (3.9 mg/kg) containing 0.86 mg/l NPFA. Peak 1 = NPFA

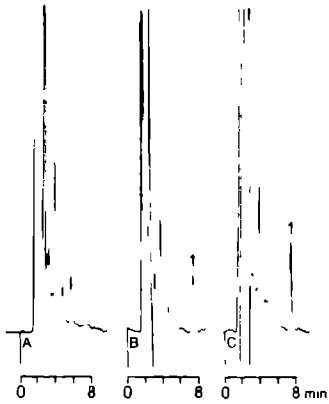


Fig. 3. Chromatograms of (A) drug-free dog urine, (B) dog urine spiked with 0.57 mg/l NPFA, (C) dog urine sample taken after intravenous administration of 5-hydroxydantrolene (3.9 mg/kg) containing 0.84 mg/l NPFA. Peak 1 = NPFA.

of the determinations in plasma and urine, measured within the same ranges at three concentrations in quadruplicate, was within acceptable limits (coefficients of variation always less than 7.6%). The mean (±S.D.) recovery for plasma was 83±5%.

When dantrolene and its reduced and oxidized metabolites were introduced into this HPLC system, all compounds could be separated within ca. 30 min. Because of band broadening, however, low concentrations escaped detection. This implies that for pharmacokinetic purposes the method as such is inadequate to determine the five compounds simultaneously. Other refinements, such as gradient elution, might favour the sensitivity.

In a preliminary survey of canine and human urine collected after administration of dantrolene and 5-hydroxydantrolene, NPFA was found in low concentrations. Total urinary recovery of this metabolite accounted for a few per cent of the dose, contributing accordingly to the mass balance of dantrolene in humans and dogs. Since in vitro hydrolysis of the azomethine bond results in aldehyde formation, urine samples were also screened for the presence of 5-(*p*-nitrophenyl)furfural. By increasing the concentration of acetonitrile in the mobile phase to 50%, the otherwise unchanged HPLC system could measure this compound (kindly supplied by Norwich Eaton Pharmaceuticals) in the spiked urine ($t_R$=6.3 min). However, in neither human nor dog samples was the aldehyde detected.

When the method described for plasma was followed, NPFA was not detected in the human samples. In pilot studies with dantrolene and 5-hydroxydantrolene administered as the parent drug in dogs, NPFA plasma concentrations in excess of the parent drug were measured, indicating that this metabolic

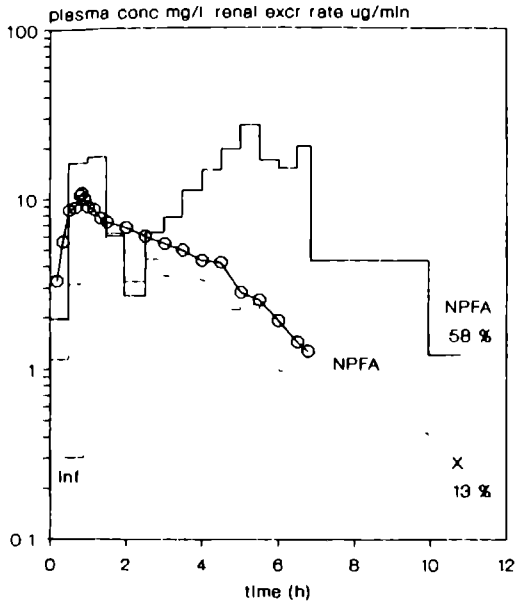


Fig 4 Plasma concentration-time curve and renal excretion rate-time profile of NPFA following its intravenous administration (0.9 mg/kg) in a dog. The infusion time (inf.) was 45 min. X = unknown metabolite.


Fig 5 Chromatogram of dog urine sample taken after intravenous administration of NPFA (0.9 mg/kg) containing 3 7 mg/l NPFA Peaks. 1 = NPFA; 2 = unknown metabolite.

pathway is important in the dog. The possibility that NPFA was formed in vitro following acidification of the plasma samples was ruled out by the absence of NPFA in standard solutions treated similarly.

The method described was also used in a metabolite kinetic experiment with NPFA administered as the parent drug in the dog. Fig. 4 shows the plasma concentration–time curve and the renal excretion rate–time profile of NPFA after its intravenous administration. The plasma curve shows non-linearity for the dose administered, with a terminal half-life of 1.4 h. When administered as the parent drug, the major excretory product found in the urine was unchanged NPFA (58% of the dose). An unknown metabolite (Fig. 5), not present in plasma, accounted for a further 13% recovery (calculated as NPFA). This unknown metabolite was absent in the samples obtained after dantrolene c.q. 5-hydroxydantrolene administration. Total body clearance ($D$/AUC) and apparent renal clearance ($A_e$/AUC) of NPFA were 6.2 and 3.6 ml/min, respectively, where $D$ is the dose administered, AUC is the area under the plasma concentration–time curve, and $A_e$ is the total amount excreted in the urine as NPFA. The apparent renal clearance of NPFA was dependent on the pH of the urine in the 5–7 range. This dependency may explain the unusual renal excretion rate–time course measured during the first 5 h of the experiment.

In conclusion, we have developed an HPLC method that is applicable to pharmacokinetic studies of dantrolene, in particular to metabolite kinetic studies in the dog.

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# SECTION III

# PHARMACOKINETICS OF BACLOFEN

Chapter 7

# COMPARISON OF THE PHARMACOKINETICS OF INTRAVENOUSLY ADMINISTERED rac-BACLOFEN AND ITS (-)-(R)- AND (+)-(S)-ENANTIOMERS IN DOGS

# COMPARISON OF THE PHARMACOKINETICS OF INTRAVENOUSLY ADMINISTERED rac-BACLOFEN AND ITS (-) (R)-AND (+)-(S)-ENANTIOMERS IN DOGS[1]

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## Summary

Baclofen is a centrally acting muscle relaxant marketed as the racemate. Since only the (-)-(R)-enantiomer is pharmacologically active, the pharmacokinetics of rac-baclofen and its enantiomers were studied individually in the same group of dogs to determine if there was any stereospecificity in the drug's kinetics after a single intravenous dose. High-pressure liquid chromatography was used to determine concentrations in plasma and urine. A major difference was found in the urinary recovery of the unchanged drug. Only about 50% of the dose of the clinically used racemate appeared as unchanged drug in the urine; whereas the active (-)-(R)-isomer was for the most part renally excreted (85%). Irrespective of isomeric composition, the renal clearance was dependent upon the creatinine clearance. Differences in non-renal clearance could not be explained by stereoselective formation of the $\beta$-hydroxymetabolite. It is concluded that in the dog, the active enantiomer is also pharmacokinetically preferred.

## Introduction

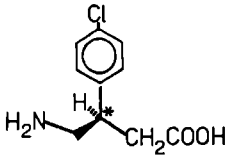
With the development of the centrally acting muscle relaxant baclofen (rac-4-amino-3-(p-chlorophenyl)-butanoic acid) in the late 1960s, a drug became available that was effective in the treatment of spasticity (1). Twenty years later, it is still one of the very few clinically used skeletal muscle relaxants for patients with this motor control disorder. It has also been shown to be of some value in the treatment

[1]Int J Clin Pharm Res 1989; 10: 239-46.

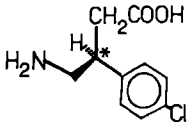
of trigeminal neuralgia (2). Baclofen is a synthetic derivative of γ-aminobutyric acid (GABA), a naturally occurring amino acid. With the introduction of the p-chlorophenyl substituent into the GABA-molecule, a centre of asymmetry is created (Fig. 1). Baclofen is marketed as the racemate, but only the (-)-(R)-enantiomer is stereospecifically active at so called $GABA_B$-receptors (3); (+)-(S)-baclofen has antagonistic properties (4). It was found that in treating trigeminal neuralgia rac-baclofen is five times less efficacious than (-)-(R)-baclofen (5).

Because of this difference in pharmacological action, it is important to elucidate whether there is also a difference between the kinetic disposition of (-)-(R)- and (+)-(S)-baclofen in the body. For rac-baclofen, renal excretion is the major route of elimination both in the dog and in man: 80-90% of the radioactivity was recovered in canine and human urine following oral or intravenous administration of the $^{14}C$-labelled drug (6). In the dog about 55% of the dose was excreted as unchanged drug; in man it was up to 75%. Less than 10% could be attributed to a pharmacologically inactive γ-hydroxymetabolite (3-(p-chlorophenyl)-4-hydroxy-butanoic acid) (6). Reduced renal function has led to high baclofen plasma levels with signs of intoxication (7,8). Other pharmacokinetic information is scarce (man) or absent (dog), probably due to the lack of suitable analytical procedures (9-11).

In the laboratory of the Department of Clinical Pharmacy, reversed-phase high-performance liquid chromatographic (HPLC) methods have been developed for the determination of this drug and its metabolite in biological fluids (12,13). By means of these methods, the pharmacokinetics of rac-baclofen, (-) (R)-baclofen, and (+)-(S)-baclofen were compared in the same group of dogs. Dogs rather than human volunteers were chosen because no data were available on the toxicity of (+)-(S)-baclofen, nor on (-)-(R)-baclofen when given as half of the racemic dose.




R(-)-baclofen




S(+)-baclofen

FIGURE 1
The enantiomers of baclofen

## DRUGS

rac Baclofen, (-)-(R)-baclofen hydrochloride, (+)-(S)-baclofen hydrochloride, and 3-(p-chlorophenyl)-4-hydroxy-butanoic acid (sodium salt) were kindly supplied by Ciba-Geigy (Basle, Switzerland). All other drugs were commercially available: pentobarbital sodium (Narcovet$^{R}$, Amerpho, Arnhem, Netherlands), mannitol, and sodium chloride solutions for injection (NPBI, Emmer Compascuum, Netherlands). Prior to administration, the baclofen dosages were dissolved in 60 ml of normal saline solution.

## ANIMAL EXPERIMENTS

Three male Beagle dogs, weighing 10-17 kg, were used. At the beginning of each experiment the animals were anaesthesized with pentobarbital sodium (30 mg/kg). Small additional doses of the anaesthetic were given during the day to keep the dogs anaesthesized for 7 h. Venous catheters were inserted into a foreleg and into the jugular vein to collect blood for sampling and to administer the drugs. A continuous infusion with a solution containing 2% mannitol in normal saline solution was given to maintain an adequate urine flow. For the collection of urine samples, a bladder catheter was introduced. After seven hours each dog was placed inside a metabolic cage to collect the spontaneously voided urine. Thereafter blood samples were taken by veinpuncture.

After an interval of approximately four weeks, the next experiment was performed. Each dog received rac-baclofen (2 mg/kg), (-)-(R)-baclofen (1 mg/kg), and (+)-(S)-baclofen (1 mg/kg) except for dog 1 who died before (+)-(S)-baclofen was administered of a cause unrelated to baclofen. The drugs were given by constant rate intravenous infusion for 3 h; the flow rate was 0.33 ml/min.

SAMPLING PROCEDURES

Blood samples of 2-3 ml were drawn at predetermined intervals for a total period of 25 h and collected in heparinized tubes. After centrifugation at 1500 g, plasma was stored at -20°C until analysis. Urine was collected quantitatively at regular intervals (approximately 30 min) during the period of anaesthesia. Care was taken to ensure that the bladder was empty at the end of each collection period. In the metabolic cages, spontaneously voided urine was collected quantitatively. The total urine collection period was 50 h. Samples of 10 ml were stored at -20°C until analysis. Urinary pH was measured in each sample.

ANALYSIS OF PLASMA AND URINE

Baclofen was measured in plasma and urine by HPLC after derivatization with o-phthaldialdehyde followed by fluorimetric detection, as previously described (12). With this method the detection limit in plasma is 0.02 mg/l. In a number of urine samples baclofen was determined with the more rapid direct-ultraviolet method which simultaneously measures the $\gamma$-hydroxymetabolite; the detection limit of both compounds is 4 mg/l (13). Plasma protein binding was measured in a number of samples by filtration through EMIT$^{R}$ free level filters (Syva, Palo Alto, USA). No aspecific drug binding to these filters was found. Creatinine was measured throughout each experiment both in plasma and urine by an automated Jaffé method.

PHARMACOKINETIC ANALYSIS

Elimination half-lives ($t_{\frac{1}{2}}$) were determined by linear regression analysis of the terminal parts of the log plasma concentration versus time curves. For the calculation of the area under the curve (AUC), the linear trapezoidal rule with extrapolation to infinity was applied. Total body clearance (CL) and apparent volume of distribution ($V_z$) were calculated according to CL = D/AUC (D being dose administered), and $V_z$ = CL.$t_{\frac{1}{2}}$/0.693. The system moment parameter mean residence time (MRT) was estimated by MRT = AUMC/AUC - 0.5T, in which AUMC is the area under the first moment of the drug concentration versus time curve and T the infusion time (14).

The apparent renal clearances ($CL_R$) were calculated in two ways An overall value of $CL_R$ was obtained by $CL_R = f_e$ CL, in which $f_e$ is the fraction of the dose excreted unchanged into the urine. The mean $CL_R$ values with standard deviation were computed from the renal excretion rate in each urine sample divided by the plasma concentration at the mid-point of the measured time interval allowing for fluctuations in the renal clearance within a dog. In the same way, mean values with standard deviation of the creatinine clearances ($CL_{CR}$) were obtained. The non-renal clearance ($CL_{NR}$) was calculated by subtracting the overall renal clearance from the total body clearance.

Data are expressed as mean ± s.d.

## Results

rac-BACLOFEN

Figure 2a shows the plasma concentration time curve and the renal excretion rate-time profile of rac-baclofen in a Beagle dog after a single intravenous infusion of 2 mg/kg. The half-life in this dog was 3.8 h, and 53.2% of the dose was excreted unchanged into the urine. Mean values of these parameters for the three dogs were 3.7(±0.3) h and 47.7 (±6.7)%, respectively. No metabolite was found in the urine. The average plasma protein binding was 35(±11)%. Values of the pharmacokinetic parameters are further presented in Table I. Mean values of the clearance in $ml.min^{-1}$ were: CL 32.1(+5.7), $CL_R$ 15.5(±3.7), $CL_{CR}$ 23(±6), and $CL_{NR}$ 16.7(±1.6).

The individual mean values of the renal parameters for each dog are listed in Table II. In this table, data of the postinfusion period are presented up to the time that a fixed sampling schedule was no longer feasible. A linear correlation was observed between the renal clearance of rac-baclofen and the creatinine clearance, their values being not significantly different (paired t-test). The renal clearance of rac-baclofen was independent of urine pH and flow.

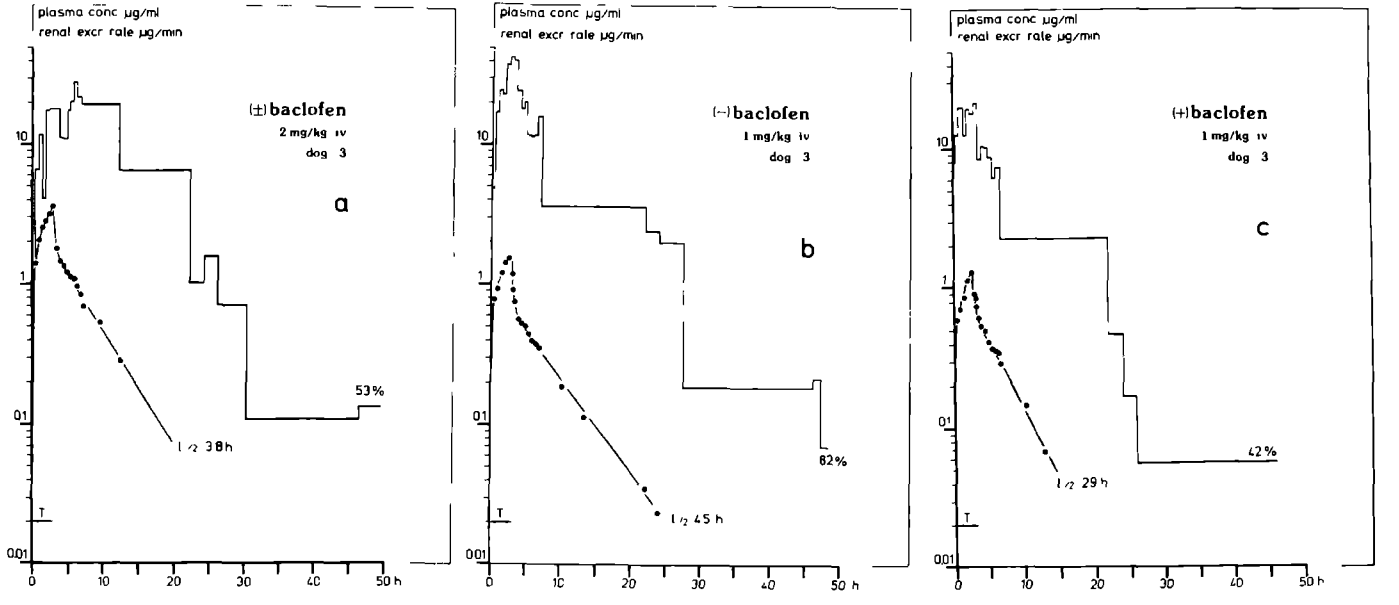



FIGURE 2

Plasma concentration-time curves and renal excretion rate-time profiles of baclofen following intravenous infusion in dog 3. % = percentage of the dose excreted unchanged into the urine; T = infusion time. a. 2 mg/kg rac-baclofen; b. 1 mg/kg (−)-(R)-baclofen; c. 1 mg/kg (+)-(S)-baclofen

TABLE I

Pharmacokinetic parameters of baclofen in dogs after intravenous infusion of 2 mg/kg rac-baclofen, 1 mg/kg (-)-(R)-baclofen, and 1 mg/kg (+)-(S)-baclofen

| Dog | Exp. | Body wt. (kg) | $t_{\frac{1}{2}}$ (h) | AUC ($mg.h.l^{-1}$) | $V_z$ ($l.kg^{-1}$) | MRT (h) | $f_e$ | CL | $CL_R$* | $CL_{CR}$** | $CL_{NR}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | ($ml.min^{-1}.kg^{-1}$) | | | |
| 1 | rac | 11.7 | 3.9 | 10.3 | 1.06 | 4.51 | 0.498 | 3.10 | 1.54 | 2.5 | 1.56 |
| 2 | rac | 10.4 | 3.4 | 11.6 | 0.79 | 3.83 | 0.402 | 2.70 | 1.09 | 1.6 | 1.61 |
| 3 | rac | 16.0 | 3.8 | 16.7 | 0.66 | 4.04 | 0.532 | 2.00 | 1.07 | 1.4 | 0.94 |
| mean | | | 3.7 | 12.9 | 0.83 | 4.13 | 0.477 | 2.60 | 1.23 | 1.8 | 1.37 |
| ± s.d. | | | 0.3 | 3.4 | 0.20 | 0.35 | 0.067 | 0.56 | 0.27 | 0.6 | 0.38 |
| 1 | (-)(R) | 11.8 | 4.3 | 7.60 | 0.81 | 5.75 | 0.875 | 2.19 | 1.92 | 1.6 | 0.27 |
| 2 | (-)(R) | 10.6 | 4.1 | 4.60 | 1.33 | 3.86 | 0.849 | 3.74 | 3.17 | 4.8 | 0.57 |
| 3 | (-)(R) | 17.3 | 4.5 | 7.83 | 0.80 | 4.27 | 0.821 | 2.04 | 1.67 | 2.8 | 0.36 |
| mean | | | 4.3 | 6.68 | 0.98 | 4.63 | 0.848 | 2.65 | 2.25 | 3.1 | 0.40 |
| ± s.d. | | | 0.2 | 1.80 | 0.31 | 0.99 | 0.027 | 0.94 | 0.81 | 1.6 | 0.15 |
| 2 | (+)(S) | 10.5 | 2.6 | 3.64 | 1.10 | 2.87 | 0.681 | 4.95 | 3.37 | 4.0 | 1.58 |
| 3 | (+)(S) | 15.5 | 2.9 | 5.72 | 0.80 | 3.22 | 0.419 | 3.17 | 1.33 | 2.3 | 1.84 |

*$CL_R = f_e.CL$; **$CL_{CR}$ = total mean value.

( )-(R)-BACLOFEN

Figure 2b shows the pharmacokinetic profile of (-)-(R)-baclofen in the same Beagle dog after a single intravenous infusion of 1 mg/kg. The half-life was 4.5 h, and it was for the greater part (82.1%) excreted unchanged into the urine. Mean values of these parameters for the three dogs were 4.3(±0.2) h and 84.8(±2.7)%, respectively. No metabolite was found in the urine. The average plasma protein binding was 18(±11)%. The pharmacokinetic parameters are further presented in Table I. Mean values of the clearances in ml.min $^{-1}$ were: CL 33.6(±7.0), $CL_R$ 28.4(±5.5), $CL_{cR}$ 39 (±18), and $CL_{NR}$ 5.17(±1.68).

The individual mean values for each dog of $CL_R$, $CL_{cR}$, urine flow, and urine pH listed in Table II were obtained in the same way as described under rac-baclofen. In all three dogs a linear correlation was found between the $CL_R$ of (-)-(R)-baclofen and the $CL_{cR}$. In two dogs the renal clearances were not significantly different from the $CL_{cR}$ (paired t-test), in one dog the $CL_R$ was slightly lower. No correlation between urine flow or pH and the $CL_R$ of (-)-(R)-baclofen was discerned.

(+)-(S)-BACLOFEN

The pharmacokinetic profile of (+)-(S)-baclofen in the same Beagle dog after a single intravenous infusion of 1 mg/kg is given in Fig. 2c. The half-life of (+)-(S) baclofen in this dog was 2.9 h, while only 41.9% of the dose was found excreted in the urine. In Table I, the same parameters for the other dog are presented. As only two dogs remained for the experiment with the (+)-(S)-isomer, no mean values are given. No metabolite was found in the urine. The average plasma protein binding was 23(±12)%. Values for the other pharmacokinetic parameters are listed in Table I.

The renal parameters are summarized in Table II. A linear correlation was found between the $CL_R$ of (+)-(S)-baclofen and the $CL_{cR}$. In one dog the $CL_{cR}$ was slightly lower, in the other dog slightly higher than the renal clearance of (+)-(S)-baclofen. Only with urinary flow rates below 0.2 ml.min $^{-1}$, did the creatinine clearance as well as the renal clearance of (+)-(S)-baclofen become flow dependent. The $CL_R$ of (+)-(S)-baclofen was independent of pH.

PHARMACOKINETIC DIFFERENCES BETWEEN rac , (-)-(R)-, AND (+)-(S)-BACLOFEN

The fraction of the dose excreted unchanged into the urine was nearly twice as large for (-)-(R)-baclofen as for the racemic mixture. The non-renal clearance of rac-baclofen was nearly 3 5 times that of the (-)-(R)-enantiomer. The half life of ( ) (R)-baclofen was slightly longer than that of rac baclofen. As this last parameter is calculated from plasma concentration data approaching the detection limit of the assay, the relevance of this finding is limited. No other significant differences were found between rac-baclofen's and (-)-(R)-baclofen's kinetic parameters as listed in Table I. When the pharmacokinetic behaviour of (+)-(S)-baclofen and (-)-(R)-baclofen is directly compared, the difference in non-renal clearance is the most striking finding. The three- to five-fold higher $CL_{NR}$ value of (+)-(S)-baclofen deserves further study.

TABLE II

Values of the parameters characterizing the renal handling of baclofen in dogs calculated from postinfusion data (3.5-7h)

| Dog | Experiment | $CL_R$* | $CL_{CR}$ | Urine flow | Urine pH |
|---|---|---|---|---|---|
| | | (ml min $^{-1}$) | | | |
| 1 | rac-baclofen | 26 ± 3 | 28 ± 4 | 0.39 ± 0.08 | 8.7 ± 0.3 |
| 2 | rac-baclofen | 17 ± 10 | 15 ± 8 | 0.24 ± 0.16 | 8.8 ± 0.1 |
| 3 | rac baclofen | 16 ± 9 | 21 ± 11 | 0.67 ± 0.24 | 8.9 ± 0.2 |
| 1 | (-)-(R)-baclofen | 14 ± 5 | 15 ± 5 | 0.41 ± 0.25 | 8.2 ± 0.3 |
| 2 | (-)-(R) baclofen | 44 ± 6 | 45 ± 4 | 0.35 ± 0.15 | 5.4 ± 0.2 |
| 3 | (-)-(R)-baclofen | 34 ± 7 | 49 ± 9 | 0.81 ± 0.38 | 7.1 ± 0.3 |
| 2 | (+)-(S)-baclofen | 47 ± 17 | 40 ± 16 | 0.12 ± 0.06 | 6.0 ± 0.5 |
| 3 | (+)-(S)-baclofen | 23 ± 5 | 30 ± 4 | 0.23 ± 0.08 | 8.9 ± 0.3 |

Data are mean ±s.d.

*$CL_R$ = renal excretion rate/plasma concentration.

## Discussion

In agreement with previous studies (6), about 50% of a dose following intravenous administration of rac-baclofen appeared as unchanged drug in the urine of dogs. When only the pharmacologically active ( )-(R)-enantiomer was administered, the drug was for the most part renally excreted unchanged (85%), provided chiral inversion did not occur (19). The $\gamma$ hydroxymetabolite was never detected which does not mean that a small percentage (2-10%) could not have been present considering the detection limit of the assay (13). Taking into account the earlier mentioned study with the $^{14}C$-labelled drug in which approximately 90% of total activity was renally recovered with less than 10% in the form of the $\gamma$-hydroxymetabolite (6), the mass balance of (-) (R) baclofen is almost complete.

The difference in renal excretion cannot be explained as just differences in renal clearance. Although the mean overall renal clearance of the (-)-(R)-enantiomer was higher than that of the racemate, so was the creatinine clearance. When the data of the experiment with (+)-(S) baclofen are also considered, it can be seen that the overall renal clearances of both enantiomers are practically equal (Table I). No matter which of the three drugs was administered, the renal clearance was always dependent upon and not very different from the creatinine clearance, at least during the postinfusion period while the dog was under anaesthesia (Table II). As glomerular filtration is a passive process and the differences in unbound baclofen plasma concentrations were small, it is not surprising that this dependency is not stereoselective. Similar renal elimination kinetics have been found for the GABA-derivative vigabatrin (rac $\gamma$-vinyl-GABA): namely, equal renal clearances of the enantiomers with equal dependency upon the creatinine clearance, and unequal recovery of unchanged drug in the urine (15, 16).

A clear correlation does not exist between the renal clearance and the parameters for passive tubular reabsorption, urine pH and urine flow, for racemic baclofen or for the separate stereoisomers. Only at very low flow rates did the renal clearance (and the creatinine clearance) become flow dependent. It is not known whether active transport processes, which are more likely to show enantiomeric differences, play

a role in the renal elimination of baclofen. As an organic zwitterion, baclofen could be a substrate for the organic anion (inhibited by probenecid) and cation (inhibited by cimetidine) tubular secretion systems (17). As a derivative of an endogenous amino acid baclofen could be actively reabsorbed in the proximal tubule (18). Stereospecificity in renal transport of amino acids does exist; for organic cations it is likely; for organic anions it is unknown. Preliminary results of experiments with probenecid were not conclusive about the organic anion secretory pathway for baclofen due to large differences in creatinine clearance.

Stereoselective metabolic clearance has been described for a number of drugs (19). β-Blocking agents and non-steroidal anti-inflammatory drugs are being investigated more and more frequently in this respect. It has been found that oxidation and glucuronidation are among the metabolic pathways that can be enantioselective. Stereoselective oxidative desamination was recently reported for primaquine (20). Also for the earlier mentioned drug vigabatrin, a marked difference in the non-renal clearance of its enantiomers could be calculated from the data presented (15). The difference in the non-renal clearance of the enantiomers of baclofen cannot be ascribed to stereoselective oxidative desamination resulting in formation of the γ-hydroxymetabolite, as this metabolite was not found in the urine. Stereoselective glucuronidation is also highly unlikely, as no glucuronides were found with the earlier described deglucuronidation procedures (13). The present findings suggest the existence of still unknown stereoselective metabolic pathways for baclofen in the dog

The pharmacologically active (-)-(R) enantiomer of baclofen is also kinetically preferred as it is for the most part renally excreted as the unchanged drug (21). As enantioselective pathways can be species dependent (22), this study should be repeated in man. The first results of a very recently published study on enantioselective determination of baclofen in human urine did show a R/S ratio in favour of the active isomer following administration of the racemate (23).

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Chapter 8

# PLASMA AND URINARY EXCRETION KINETICS OF ORAL BACLOFEN IN HEALTHY SUBJECTS

# PLASMA AND URINARY EXCRETION KINETICS OF ORALLY ADMINISTERED BACLOFEN IN HEALTHY SUBJECTS[1]

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**Summary**

Baclofen, a centrally acting muscle relaxant, is used in the treatment of spasticity. Its pharmacokinetics has been derived from plasma and urine data in four healthy subjects, whose renal function was simultaneously measured.

After oral administration of a single 40 mg dose, baclofen was mainly excreted unchanged by the kidney, 69 (14) %. The half-life, calculated from extended least squares modelling (ELSMOS) both of plasma and urine data was 6.80 (0.68) h, which is longer than reported in most studies based solely on plasma data.

The renal excretion rate constant had the high mean value of 0.35 (0.24) $h^{-1}$, and the apparent renal clearance of baclofen equalled the creatinine clearance. Passive tubular reabsorption is relatively unimportant, since no dependence was observed on variables urinary flow or pH.

Although active tubular secretion may contribute to its renal clearance, as shown by the effect of co-administration of probenecid, glomerular filtration appears to be the dominant transport mechanism.

**Key words:** baclofen; renal function, healthy subjects, pharmacokinetics, probenecid, tubular secretion

[1]Eur J Clin Pharmacol 1989; 37: 181-4.

Baclofen (rac β-[p-chlorophenyl]-GABA), a centrally acting muscle relaxant, is used in the management of patients with spastic disorders [1]. In 1972 Faigle and Keberle [2] first described the disposition of $^{14}$C-baclofen after a single oral dose in man; 80% of the radioactivity was recovered in urine, mainly as unchanged drug. In a more recent report, 60% of a dose of baclofen was excreted unchanged by the kidney, with a mean overall renal clearance of 100 ml.min$^{-1}$ [3]. In a study in dogs, the renal clearance of baclofen was found to approximate the creatinine clearance [4].

The elucidation of the renal elimination mechanism for baclofen is important because patients with diminished renal function may develop baclofen toxicity, which is manifested by hypothermia, bradycardia, hypotension, blurred vision, mental changes, myoclonus, or abnormal EEG patterns [5-7]. In elderly patients with lower creatinine clearance due to their age, an unacceptably high level of drowsiness occurred with relatively low doses of the drug [8]. Therefore the pharmacokinetics of baclofen has been studied, as derived both from plasma and urine data in healthy subjects, whose renal function was simultaneously measured.

## Materials and methods

Tablets containing rac-baclofen 10 mg were obtained commercially (Lioresal®, Ciba-Geigy). Probenecid was obtained as 500 mg tablets (Benemid®, Merck Sharp & Dohme).

### STUDY DESIGN

The subjects were four healthy Caucasian volunteers (1 male and 3 females), ranging from 25 to 42 years, and with a mean (SD) body weight of 69 (9) kg. They had agreed to participate in the study. The subjects did not take any other medication. Each subject received a single oral dose of 40 mg baclofen after a standard breakfast. Serial 1-2 ml blood samples obtained by fingertip puncture were collected in heparinised tubes at predetermined intervals for a total period of 24 h. Plasma was separated by centrifugation (1500 g) and stored at -20°C until analysed. Urine was collected by spontaneous voiding for 50 h The total

volume and pH of each specimen was measured and 10-ml samples were stored at -20°C until analysed.

In two subjects, the experiment was repeated with probenecid 0.5 g to a total dose of 3 g as co-medication.

## ANALYSIS OF PLASMA AND URINE

rac-Baclofen in plasma and urine was measured by HPLC with fluorimetric detection [9]. High urinary baclofen concentrations were determined by the rapid direct-UV method [10]. Plasma protein binding in a number of plasma samples was measured by filtration through EMIT Free level filters (Syva, Palo Alto CA, USA). No nonspecific binding to the filters was found. Plasma probenecid concentrations ($C_{prob}$) were determined by HPLC [11]. Creatinine both in plasma and urine was measured by an automated Jaffé method.

## PHARMACOKINETIC AND STATISTICAL ANALYSIS

Combined plasma and urine data were used for compartmental analysis according to the model shown in Fig.1. Curve fitting and calculation of the rate constants were done with an extended least squares modelling system (ELSMOS), which automatically weights the data [12]. The first order rate constants are defined as: $k_{12}$ and $k_{21}$ are the transfer rate constants from the central (1) to the peripheral (2) compartment and vice versa, $k_a$ is the absorption rate constant, $k_e$ and $k_{res}$ are the corresponding rate constants for unchanged drug appearing in the urine and the remaining processes. The other parameters obtained are the volume of the central compartment ($V_c/f$), the disposition rate constants $\lambda_1$ and $\lambda_2$, and their corresponding half-lives $t_{\frac{1}{2}\lambda_1}$ and $t_{\frac{1}{2}}$.

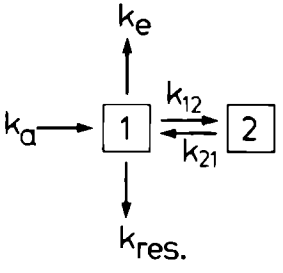


FIGURE 1
Schematic representation of the pharmacokinetic model used to calculate the rate constants

Apparent renal clearance ($CL_R$) values were computed from the renal excretion rate in each urine sample divided by the plasma concentration at the mid-point of the measured interval. Creatinine clearances ($CL_{cr}$) were similarly obtained.

Data are expressed as mean (SD). Statistical significance was tested by t-tests, paired if appropriate; $p < 0.05$ was taken to indicate significance.

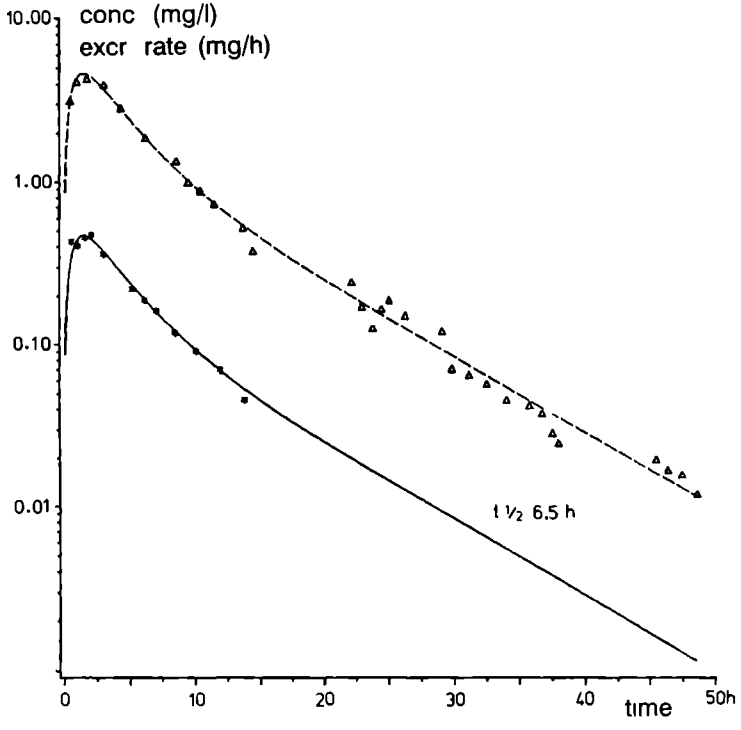


FIGURE 2

Plasma concentration-time (——) and renal excretion rate-time (---) curves of baclofen in a healthy subject after a single oral dose of 40 mg. ELSMOS-computed curves from combined plasma and urine data fitted to the model shown in Fig.1. * = measured plasma concentration, Δ = 'vertical' mid point of the measured renal excretion rate interval

The fitted plasma concentration-time and renal excretion rate-time curves of baclofen in a healthy subject after a single oral dose of 40 mg are shown in Fig.2; the computed half-life in this subject was 6.48 h. Similar profiles were found in the other subjects. Individual values of all the calculated pharmacokinetic parameters are given in Table 1. The average plasma protein binding was 31 (11) %. The apparent renal clearance of baclofen was high and was found to equal the creatinine clearance ($p > 0.05$). Individual mean values of renal function for each subject are listed in Table 2. $CL_R$ was independent of urine pH and flow. The average recovery of unchanged drug in the urine was 69 (14)%.

The results of the experiments with probenecid are illustrated in Fig.3. In both subjects the apparent renal clearance of baclofen after probenecid was significantly lower ($p < 0.001$) than in its absence (Table 2). However, the creatinine clearance was also lower in the experi-

TABLE 1

Pharmacokinetic parameters of baclofen in healthy subjects derived from combined plasma and urine data after a single 40 mg oral dose

| Parameter | | Subject | | | | Mean (SD) | |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | | |
| $k_{12}$ | ($h^{-1}$) | 0.073 | 0.194 | 0.125 | 0.440 | 0.208 | (0.162) |
| $k_{21}$ | ($h^{-1}$) | 0.118 | 0.177 | 0.188 | 0.171 | 0.163 | (0.031) |
| $k_e$ | ($h^{-1}$) | 0.340 | 0.148 | 0.222 | 0.692 | 0.351 | (0.241) |
| $k_{res}$ | ($h^{-1}$) | 0.107 | 0.154 | 0.092 | 0.152 | 0.126 | (0.031) |
| $k_m$ | ($h^{-1}$) | 0.618 | 0.677 | 2.09 | 0.339 | 0.932 | (0.788) |
| $V_c/f$ | (l) | 27.5 | 58.5 | 35.0 | 14.4 | 33.9 | (18.5) |
| $\lambda_1$ | ($h^{-1}$) | 0.541 | 0.581 | 0.512 | 1.35 | 0.745 | (0.403) |
| $\lambda_2$ | ($h^{-1}$) | 0.097 | 0.092 | 0.115 | 0.107 | 0.103 | (0.010) |
| $t_{1/2\lambda_1}$ | (h) | 1.28 | 1.19 | 1.35 | 0.514 | 1.09 | (0.39) |
| $t_{1/2}$ | (h) | 7.12 | 7.56 | 6.02 | 6.48 | 6.80 | (0.68) |

ments with probenecid. In Subject 3 $CL_R$ was not significantly different from $CL_{CR}$ ($p > 0.50$) irrespective of the presence or absence of probenecid. In the other subject (4), $CL_R$ was significantly lower than $CL_{CR}$ ($p < 0.001$) in the presence of probenecid.

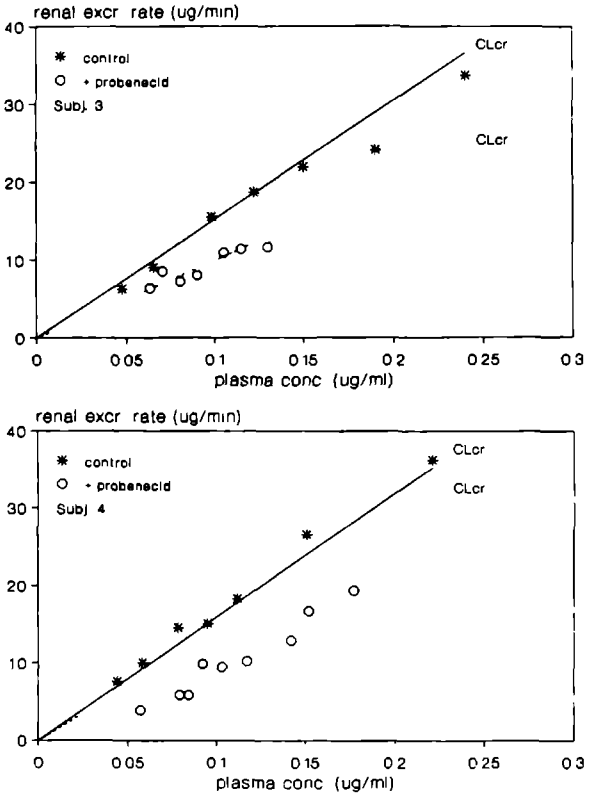


FIGURE 3

Relationship between the renal excretion rate and the plasma concentration of baclofen in two subjects. The lines represent mean $CL_{CR}$ values, with (---) and without (——) probenecid, the markers are calculated $CL_R$ values of individual samples. Note that in one subject (upper) the markedly reduced creatinine clearance in the experiment with probenecid may explain the reduction in the renal clearance; in the other subject (lower) active tubular secretion appears to contribute to glomerular filtration

TABLE 2

Renal handling of baclofen in healthy subjects

| Subject | Exp | $f_e$ | $CL_R$ | $CL_{CR}$ | Urine flow | Urine pH | $C_{prob}$ |
|---|---|---|---|---|---|---|---|
| | | | ($ml.min^{-1}$) | | | | ($mg.l^{-1}$) |
| 1 | b | 0.756 | 133 (49) | 137 (30) | 1.8 (0.4) | 5.3 (0.5) | |
| 2 | b | 0.498 | 147 (18) | 150 (16) | 1.6 (0.4) | 5.6 (0.3) | |
| 3 | b | 0.661 | 142 (11) | 152 (22) | 0.8 (0.4) | 6.0 (1.1) | |
| 4 | b | 0.832 | 170 (9) | 159 (19) | 0.8 (0.2) | 5.4 (0.9) | |
| 3 | b+p | 0.627 | 101 (14) | 99 (12) | 3.1 (1.7) | 7.6 (0.8) | 104 (25) |
| 4 | b+p | 0.539 | 90 (17) | 140 (16) | 1.6 (0.8) | 5.9 (0.7) | 69 (18) |

calculated from data beyond 4 h following drug intake, except $f_e$; $f_e$ = fraction of dose excreted unchanged in urine; b = baclofen, p = probenecid.

## Discussion

The present data confirm earlier studies [2,3,13-15] showing that renal excretion is an important pathway in the elimination of baclofen from the human body. The rate of renal excretion was fast and it governed the elimination process in three of the four subjects studied. In Subject 4, who had the highest $k_e$, the urinary mass balance could be considered to be complete as the missing part was expected to be present in the faeces. In the other subjects, there must have been further pathways of elimination, such as biotransformation to the γ-hydroxymetabolite [2], although this substance was not detected. In these subjects incomplete absorption is an unlikely explanation for the smaller amount recovered in urine, since almost no difference has been found in the urinary recovery of total radioactivity after intravenous or oral administration of baclofen [13].

The urinary excretion curve clearly shows a final elimination phase, which was much more difficult to discern in data obtained solely from plasma measurements. This observation may, at least in part, explain the earlier reported variation in the half-life of baclofen, since the beginning of the final elimination phase happens to coincide with the detection limit of the assay in plasma. From the combined plasma and urine data, a longer mean half-life (6.8 h) was calculated than that previously calculated just from plasma concentrations (average 2.0 to 4.4 h depending upon the study) [3,8,16]. Only Krauss [17] reported a similar, prolonged half-life of 6.9 h following 20 mg p.o. The mean value she calculated for $t_{\frac{1}{2}\lambda_1}$ (0.95 h) also agrees with the value found in the present study. The method of computing employed here may explain why $k_a$ was found to be only half the value she presented. Since primary interest lay in the elimination of baclofen, no attempt was made to correct for the delay caused by the inclusion of urine data.

As observed in the previous experiments in dogs, the apparent renal clearance of baclofen was found to be equal to the creatinine clearance. It is anticipated that, like the findings in dogs, the dependence upon the glomerular filtration rate (GFR) will not be stereoselective, provided that the difference between the unbound plasma concentrations of the baclofen enantiomers is just as small. Since none of the subjects showed dependence of kinetics on urine flow or pH, it is

expected that passive tubular reabsorption is a relatively unimportant process. Taking into account the fact that the creatinine clearance slightly overestimates the GFR in healthy subjects [18], and that approximately 30% of baclofen is bound to plasma proteins, the intrinsic renal clearance of baclofen might be higher than the GFR, suggesting active secretion in addition to passive filtration. The reduced renal clearance found in the presence of probenecid seems to support this hypothesis, but the differences seen in the creatinine clearance are a complicating factor. The influence of probenecid is clearly seen in the complete urine mass balance for Subject 4 (Fig.3, lower graph): the renal clearance was reduced, both in comparison to its value without probenecid and relative to the creatinine clearance in the same experiment. In Subject 3, on the other hand, the markedly reduced creatinine clearance in the experiment with probenecid may explain the lowered renal clearance of baclofen. Apparently the contribution of filtration to the clearance process was of major importance, and masked other potential secretory pathways in this subject, whose renal excretion alone could not totally account for the elimination of baclofen. Incomplete administration of probenecid was ruled out by serial measurements of its plasma concentration (Table 2) [11].

The present study demonstrates that urinary excretion rate data aid in understanding the pharmacokinetics of baclofen in man. It was found that although the mechanism of urinary excretion may be more complicated than just glomerular filtration, there is a high correlation between the apparent renal clearance of baclofen and the creatinine clearance.

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Chapter 9

# PHARMACOKINETICS OF BACLOFEN IN SPASTIC PATIENTS RECEIVING MULTIPLE ORAL DOSES

# PHARMACOKINETICS OF BACLOFEN IN SPASTIC PATIENTS RECEIVING MULTIPLE ORAL DOSES[1]

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## Abstract

The pharmacokinetics of racemic baclofen as determined from plasma and urine data in six spastic patients treated with individualized oral doses, 30-80 mg daily, are presented. Peak plasma concentrations were achieved 1.9 h (± 0.7) after a dose. The fluctuation in the plasma concentration was great, ranging from 188 to 439%. The total body clearance averaged 175 ml.min$^{-1}$ (± 44), plasma protein binding 35% (± 6). Baclofen was for the greater part excreted unchanged by the kidney, 65% (± 16). Its apparent renal clearance equalled the creatinine clearance. The contribution of the renal clearance to the total body clearance can explain the previously described toxicity when renal impairment is present. The results agree with earlier reports on single doses in healthy subjects.

**Key words:** Baclofen; Clearance; Patients; Pharmacokinetics

## Introduction

The gamma-aminobutyric acid (GABA) agonist baclofen is a centrally acting muscle relaxant, which is used in the treatment of spasticity. Once initiated, therapy with this drug is often continued for a prolonged period of time. Until now, pharmacokinetic studies with baclofen have mostly dealt with single doses in healthy subjects [1-4]. Multiple-dose studies in patients are essential in order to obtain pharma-

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[1]Pharm Weekbl [Sci] 1990; 12: 71-4.

cokinetic information in the same circumstances in which the drug is routinely used. In a multiple-dose study in elderly patients receiving baclofen in a fixed dosage schedule, the investigation had to be discontinued as most patients developed severe drowsiness [5]. In this communication we report the results of a pharmacokinetic study of baclofen in spastic patients, while they were being treated with individualized oral dosages.

## Methods

### PATIENTS

The subjects were 6 neurological in-patients (4 males and 2 females) with spasticity of spinal or cerebral origin. Their mean (± SD) age and body weight were 52 years (± 6) and 83 kg (± 13), respectively. None was overtly incontinent for urine. Only 1 patient was without any co-medication; a variety of drugs was taken by the other patients. Full details are given in Table 1. The patients were treated with oral racemic baclofen (Lioresal® tablets, Ciba-Geigy, Arnhem, the Netherlands) in doses varying between 30 and 80 mg daily given at 6-h or 8-h intervals (Table 2). The duration of baclofen medication varied among the patients, but was such to have caused steady-state in each patient for the particular dosage regimen reported.

### SAMPLING PROCEDURE

Serial 2-3 ml venous blood samples were collected in heparinized tubes at predetermined times for the period of a dosage interval. Plasma was separated by centrifugation (1,500 g) and stored at -20°C until analysed. Urine was collected on spontaneous voiding in portions equalling the dosage intervals for a period of 3-4 intervals. The total volume and pH of each urine specimen was measured and 10 ml samples were stored at -20°C until analysed. The total number of urine specimens obtained from the 6 patients was 21.

TABLE 1

Characteristics of the patients

| Patient | Age/gender | Body weight (kg) | Clinical diagnosis | Co-medication |
|---|---|---|---|---|
| A | 49/M | 107 | spinal cord injury; back pain | lactulose, methenamine mandelate |
| B | 47/F | 82 | multiple sclerosis | vitamines B |
| C | 60/M | 80 | multiple sclerosis | co-trimoxazole, phenprocoumon, triamterene, vitamine B1 |
| D | 52/F | 71 | normal pressure hydrocephalus; radicular syndrome; lumbago; hypothyroidism | oxazepam, thyroxine sodium |
| E | 58/M | 77 | cerebrovascular insufficiency; polyneuropathy; epilepsy; dementia | phenytoin |
| F | 46/M | 80 | multiple sclerosis | |

## ANALYSIS OF PLASMA AND URINE

Racemic baclofen in plasma and urine was measured by high pressure liquid chromatography (HPLC) with fluorimetric detection [6]. The minimum detectable concentration was 0.02 $mg.l^{-1}$. High urinary baclofen concentrations were determined, using the rapid direct UV method [7]. Plasma protein binding in a number of plasma samples was measured at room temperature by filtration through EMIT® Free level filters (Syva, Palo Alto, USA). No nonspecific binding to the filters was found. Creatinine both in plasma and urine was measured by an automated Jaffé method.

## ANALYSIS OF DATA

Minimum ($C_{ss(min)}$) and maximum ($C_{ss(max)}$) plasma concentrations, and the time to reach the maximum ($t_{max}$) were obtained from the data at the sampling times. The fluctuation in the plasma concentration was expressed as $C_{ss(max)}/C_{ss(min)}.100\%$. From the area under the curve during a dosage interval $\tau$ ($AUC_{ss(\tau)}$), which was calculated by the linear trapezoidal rule, the average steady-state plasma concentration ($C_{ss(av)}$) was computed according to $C_{ss(av)} = AUC_{ss(\tau)}/\tau$. The total body clearance (CL/f) was obtained according to $Cl/f = D/AUC_{ss(\tau)}$ in which f is the fraction of the dose systemically available and D the dose per dosage interval. Apparent renal clearance ($CL_R$) values were computed from the renal excretion rate in each urine sample divided by the average steady state plasma concentration. Creatinine clearances ($CL_{CR}$) were similarly obtained. From the total volume of urine collected, the fraction of systemically available drug excreted unchanged into the urine ($f_e$) was calculated. The nonrenal clearance ($CL_{NR}$) was obtained according to $CL_{NR} = (CL/f)(1-f_e)$.

Data are expressed as mean ($\pm$ SD). Statistical significance was tested by the paired t-test; $P < 0.05$ was taken to indicate significance.

TABLE 2

Pharmacokinetic parameters of baclofen in spastic patients following multiple oral doses

| Patient | Daily dose | τ (h) | $t_{max}$ (h) | $\frac{C_{ss(max)} \cdot 100}{C_{ss(min)}}$ % | $C_{ss(av)}$ (mg.l$^{-1}$) | $f_e$ | Clearance (ml.min$^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | CL/f | $CL_R$ | $CL_{CR}$ | $CL_{NR}$ |
| A | 40 | 6 | 3.0 | 188 | 0.180 | 0.90 | 154 | 139 | 144 | 15 |
| B | 40 | 6 | 1.1 | 209 | 0.180 | 0.74 | 154 | 115 | 121 | 40 |
| C | 80 | 6 | 2.0 | 354 | 0.336 | 0.53 | 165 | 103 | 94 | 78 |
| D | 30 | 8 | 2.1 | 220 | 0.171 | 0.46 | 122 | 56 | 56 | 65 |
| E | 60 | 8 | 2.0 | 256 | 0.191 | 0.57 | 218 | 143 | 97 | 95 |
| F | 75 | 8 | 1.1 | 439 | 0.220 | 0.71 | 237 | 169 | 146 | 68 |
| Mean | | | 1.9 | | | 0.65 | 175 | 121 | 110 | 60 |
| SD | | | 0.7 | | | 0.16 | 44 | 39 | 34 | 29 |

## Results

Figure 1 depicts the plasma baclofen concentration-time curves following multiple oral doses in 6 spastic patients during the dosage interval period. The values obtained for the pharmacokinetic parameters are listed in Table 2. Individual mean values are given for the apparent renal clearance of baclofen and for the creatinine clearance. The $CL_R$ was high and was not significantly different from the $CL_{cr}$ when all renal clearance values from the 6 patients (n = 21) were examined. No correlation between urine flow [overall mean value 1.2 ml.min$^{-1}$ ($\pm$ 0.6)] or pH [overall mean value 6.3 ($\pm$ 1.0)] and the $CL_R$ was discerned. The average plasma protein binding was 35% ($\pm$ 6) as determined in 12 samples from different patients with total baclofen plasma concentrations varying from 0.10 to 0.40 mg.l$^{-1}$.

## Discussion

The pharmacokinetic parameters of baclofen found in this study with spastic patients treated with multiple oral doses are in good agreement with the results obtained in the earlier mentioned single-dose studies in healthy subjects. Only in elderly patients (aged 69-81 years) were maximum concentrations after dosing reported at later times [5]. With both dosage regimens (q6h and q8h), the fluctuation in the plasma concentration was great - ranging from slightly less than 200% to just over 400% - though consistent with a mean half-life of 7 h as reported previously in healthy subjects [3 4]. The half-life was not determined in the present study, because this would have implied abrupt withdrawal of the drug risking seizures, hallucinations, and dyskinesias [8 9]. The clinical relevance of the wide fluctuation in the plasma concentration is not known. Therapeutic concentrations are considered to be 0.08 to 0.4 mg.l$^{-1}$ [10], while concentrations of over 0.8 mg.l$^{-1}$ have been associated with toxicity [11 12]. Cerebrospinal fluid levels may possibly reflect the concentration at the site of action of this centrally acting muscle relaxant better than plasma [13]. Liquor obtained from 3 patients in the present study, however, failed to give detecta-

ble baclofen concentrations (assayed as plasma), making it unsuitable as a sampling medium.

The apparent renal clearance of baclofen was found to be equal to the creatinine clearance. This relationship was established using renal clearance values derived from data not solely obtained during the elimination phase. The altered bladder function of patients with spasticity (see below) precludes short sampling intervals, while chronic dosing further restricts sampling possibilities. However, it can be assumed that, like the findings in healthy subjects in whom ample urine sampling during elimination revealed an identical relationship [4], the variation in baclofen's renal clearance is closely related to the variation in the creatinine clearance. The influence of the renal function on the pharmacokinetic pattern of baclofen is most clearly seen in patients D and F with almost equal nonrenal clearance values (Table 2). The difference in total body clearance can be explained completely by the difference in creatinine clearance. Thus, the 2.5-fold difference in dose is not reflected in the average steady-state plasma concentra-

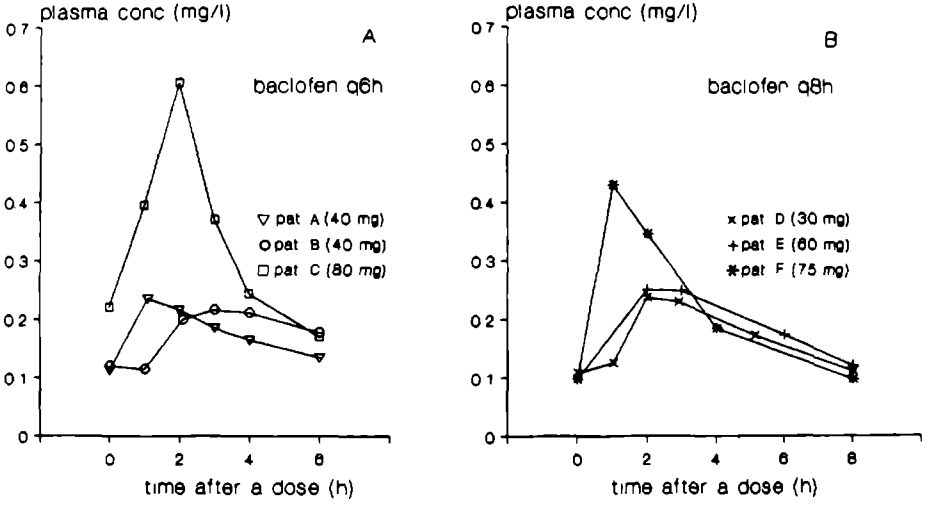


FIGURE 1

Plasma baclofen concentration-time curves following multiple oral doses q6h (A) or q8h (B) for the period of the dosage interval in 6 spastic patients

tions, which were 0.171 and 0.220 mg.l $^{-1}$, respectively. The nearly equal plasma concentration-time curves of patients D and E (Fig. 1B) with a 2-fold difference in dose, can also be ascribed for the greater part to the differences in renal function. These findings may explain the earlier reported toxicity of baclofen with normal doses in patients with renal impairment [11 14 15], and are in agreement with a case of intoxication where a previously effective dose led to unacceptably high plasma concentrations after both the creatinine clearance and the renal clearance had dropped considerably [unpublished observation].

The present study also demonstrates the practical difficulties of urine sampling in spastic patients in whom dysfunction of voiding is frequently present [16]. Reduced voluntary control and altered reflex activity imply that the short sampling intervals one may need for pharmacokinetic purposes will often not be feasible, while the volume of a portion may be compromised. Very likely there was some inaccuracy in the portions collected in patients C and E. When their renal clearance values were computed differently (by multiplying $f_e$ by CL/f ), the values were smaller (87 and 124 ml min $^{-1}$) though reasonably close to the listed values.

## Conclusion

The results of this baseline study suggest that the pharmacokinetics of multiple-dose baclofen in spastic patients will not differ greatly from those previously reported after single doses in healthy subjects, the renal function being a prominent variable. It remains to be established how the separate enantiomers of the clinically used racemic mixture will behave, but it is anticipated that the observed relationship between the renal clearance of baclofen and the creatinine clearance will not be stereoselective [4 17].

**Acknowledgements**

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# SECTION IV

# PHARMACOKINETICS OF DANTROLENE

Chapter 10

# WHOLE-BODY AUTORADIOGRAPHY OF $^{14}$C-DANTROLENE IN THE MARMOSET MONKEY

# WHOLE-BODY AUTORADIOGRAPHY OF $^{14}$C DANTROLENE IN THE MARMOSET MONKEY[1]

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Dantrolene (D) is a skeletal muscle relaxant which impairs muscle contraction by interfering with the release of calcium from the sarcoplasmic reticulum. D is used in the treatment of spasticity and in the prevention and treatment of malignant hyperthermia (Ward et al. 1986). Despite its presence on the market for nearly twenty years, only little is known about its distribution in the body. In man, the apparent volume of distribution is approximately 0.5 l.kg $^{-1}$ body weight as calculated from the data presented by Asari et al. (1984). A saliva:plasma ratio of 0.05 indicates poor distribution into saliva and may be indicative of high plasma protein binding (unpublished observation). Negligible amounts of unchanged dantrolene have been found in human urine and bile. The known metabolites of dantrolene, 5-hydroxydantrolene (5-HD) and acetylated 7-aminodantrolene (ACE-D), have been detected in human urine; their concentrations in bile were extremely low (Meyler et al. 1980; Wuis et al. 1983; Asari et al. 1984). It has been suggested that D being a lipophilic compound, may cross the blood-brain barrier (Ward et al. 1986).

In vitro studies with the $^{14}$C-labelled drug have demonstrated that D binds to the sarcoplasmic reticulum of rabbit skeletal muscle (Dehpour et al. 1982). In vivo rat studies have shown that D or one of its metabolites forms a less stable complex with the sarcoplasmic reticulum than with the hepatic endoplasmic reticulum (Roy et al. 1980); this is an important finding in the light of the drug's liver toxicity in patients undergoing chronic high dose therapy (Ward et al. 1986). Whole body fluorescent studies in mice have revealed that intraperitoneally administered D accumulates mainly in the intestines, liver, and urinary bladder, partially in the kidneys, and only slightly in the muscles

[1]Pharmacol Toxicol 1989; 64: 156-8.

(Nouhnejade & Maleki 1985). Fluorescence was probably absent in the brain, but this was not clearly documented.

The aim of the present investigation was to clarify the body distribution of D relevant for therapeutic use, i.e. at steady state in the monkey, a primate. For this purpose, $^{14}$C-D (fig. 1) was administered by intravenous infusion, and the distribution of radioactivity was studied by means of whole-body autoradiography. Prior to the actual distribution experiment, a preliminary experiment with the unlabelled drug was performed to ascertain the pharmacokinetic behaviour of D in the monkey.

$^{14}$C-DNa with a specific activity of 32.6 µCi/mg was kindly supplied by Norwich Eaton (Norwich NY, U.S A.). The chemical and radiochemical purity were both better than 98% Unlabelled DNa, 5-HD, 7-aminodantrolene (AM-D), and ACE-D were supplied by the same manufacturer. Instagel$^{R}$ was obtained from Packard, ketamine hydrochloride (Vetalar$^{R}$) from Parke Davis, and halothane (Fluothane$^{R}$) from ICI. All other chemicals were of analytical grade. Prior to administration, 3.1 mg of ($^{14}$C) DNa was dissolved in 0.4 ml of propylene glycol, 0.25 µl of 0.1 M sodium hydroxide, and water to a final concentration as mentioned below.

A male marmoset monkey (Callithrix jacchus) weighing 384 g was given 1.40 mg of unlabelled DNa by constant rate intravenous infusion for 3 hr. The infusion fluid contained 0.155 mg/ml of DNa and the infusion rate was 3.0 ml/hr. Total sampling time was 5 hr. A male marmoset monkey weighing 328 g was given 88 µCi of $^{14}$C-DNa by constant rate intravenous infusion for 5.2 hr. The infusion fluid contained 0.207 mg/ml of DNa corresponding to 6.75 µCi/ml, and the infusion rate was 2.5 ml/hr. The animals were anaesthetised with ketamine hydrochloride (40 mg/kg). During the experiment, anaesthesia was maintained with halothane. Blood samples of 0.8 ml were taken at regular time intervals and collected in heparinised tubes. After centrifugation at 1500 x g, plasma was kept at -20°C until analysis. Urine was collected quantitatively by a bladder catheter. All samples were kept protected from light.

Whole-body autoradiography was carried out according to the Ullberg method as described by Van der Kleijn (1969). The animal was instantly killed under deepened anaesthesia by submersion into isopentane, cooled with solid carbon dioxide to -80°. The whole body was embedded in a 5%

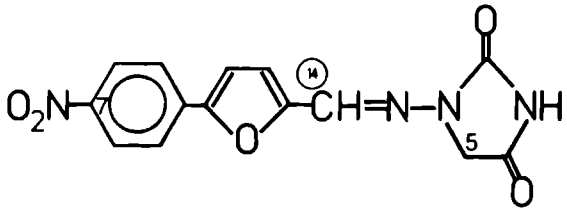

FIGURE 1
Structural formula of $^{14}$C-dantrolene

carboxymethylcellulose gel of 0°, and again cooled to -80°. By a special microtome (PMV 450, Stockholm, Sweden) at a temperature of 20° sagittal sections of 30 µm thickness were made, attached to Scotch tape no. 810. After freeze-drying for 48 hr at atmospheric pressure the sections were brought onto Structurix D7FW Rontgen film (Agfa-Gevaert). Exposure took place at -20° for a period of 6 weeks. After the films were developed positive prints were made; the white areas correspond to the presence of radioactivity.

Total radioactivity in plasma and urine was determined using a liquid scintillation counter. Samples of 0.1 ml were mixed with 11 ml of Insta-gel®. Counting was performed with a Packard Tri-Carb Liquid Scintillation Spectrometer Model 3380, using external standardisation. D and its metabolites were determined by HPLC based upon an earlier described method (Wuis et al. 1982).

Following the intravenous infusion with unlabelled D, a half-life of 0.82 hr was found. Total body clearance was 0.77 l.h$^{-1}$.kg$^{-1}$ and the apparent volume of distribution 0.91 l.kg$^{-1}$. As in man, the major metabolite appeared to be 5-HD. The mean plasma 5-HD:D ratio was 1.5. Urinary recovery of unchanged D in the 5 hr period was negligible. In this period, 6% of the dose administered was excreted as 5-HD, while only traces of AM-D and ACE-D were detected. With a half-life of less than 1 hr, steady state conditions are reached after 5 hr of linear infusion.

In the monkey experiment with $^{14}$C-D, the total radioactivity present in plasma after 5 hr, expressed as D, was 5 mg/l. This implies that all the observed radioactivity in plasma could be accounted for by D and its main metabolite. Total radioactivity recovered in the urine in the 5 hr was 7%, which corresponds to the percentage recovered as metabolites.

The autoradiograms of $^{14}$C-D in the marmoset monkey show agreement with whole body fluorescent studies in mice (Dehpour et al. 1982); the drug has unequal distribution through the body (fig. 2). A high accumulation of radioactivity was observed in the intestines, liver, gall bladder, kidneys, and urinary bladder. The presence in the intestines could be explained by biliary excretion, which is a major elimination pathway for 5-HD in the dog (unpublished observation). As the concentrations in human bile are low (Meyler et al. 1980), this pathway is

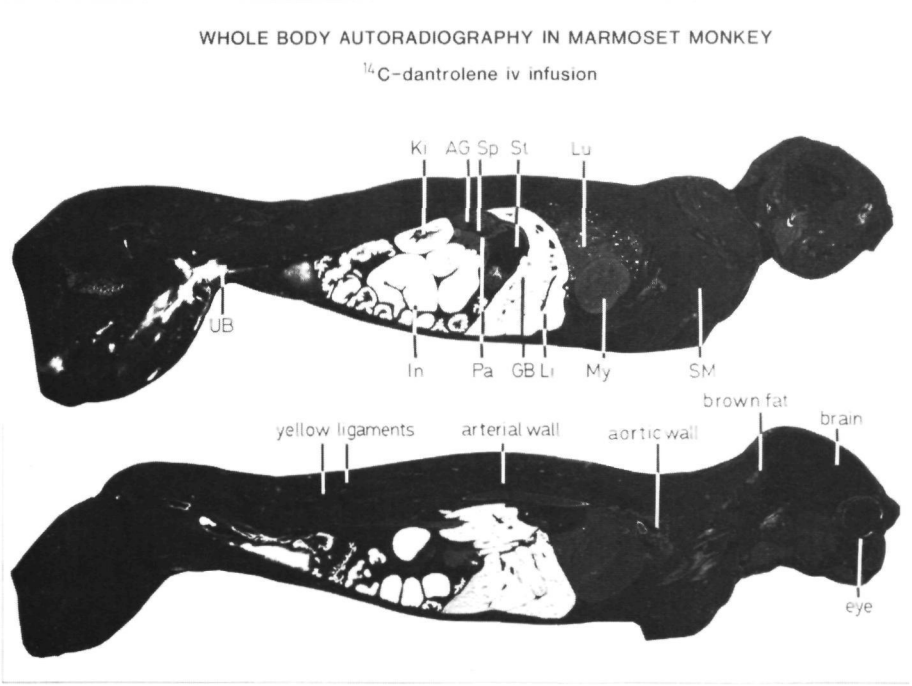


FIGURE 2
Autoradiograms showing the distribution of radioactivity (= white areas) after 5 hr of linear infusion of $^{14}$C-dantrolene in a marmoset monkey (sagittal sections at two different levels). AG = adrenal gland, GB = gall bladder, Ki = kidney, In = intestine, Li = liver, Lu = lung, My = myocard, Pa = pancreas, SM = skeletal muscle, Sp = spleen, St = stomach, UB = urinary bladder

probably less important in man. The high level of radioactivity in the liver as compared to that in skeletal muscle agrees with the binding studies in the rat (Roy et al. 1980). Presence of radioactivity in the kidneys is probably due to metabolites, as the total radioactivity recovered in the urine corresponded to the amount excreted as metabolites.

High levels of radioactivity were also found in arterial walls, in the lungs, and in the eyes. The radioactivity in the lungs could be due to either undissolved D particles or to a local precipitation of the drug. Precipitation is a reasonable explanation because in no other tissue with dense capillary circulation (liver, spleen, glandular tissue) spots of radioactivity were found. Moreover, pulmonary complications have been reported to occur in a number of patients following oral D treatment (Petusevsky et al. 1979; Miller & Haas 1984). Pulmonary embolism was suspected even though it seemed unlikely in these patients based on radiological evidence. It apparently did occur in a patient treated with nitrofurantoin, a structural analogue of D with identical toxicity on liver and lung (Israel & Diamond 1962; Faling et al. 1980). In the eye, a high level of radioactivity was found in the choroid layer and in the ciliary bodies. This finding as well as relaxation of the extraocular muscles, may explain the visual disturbances reported as a side effect of D (Dykes 1975).

In addition to skeletal muscle, considerable levels of radioactivity were found in the myocard, spleen, pancreas, adrenal glands, yellow ligaments, and brown fat. Only traces of radioactivity were found in the brain. This observation suggests that no significant passage of the blood-brain barrier occurs. This holds for the intact D molecule. Cleavage of the azomethine bond has been found to occur, at least in vitro (Inotsume & Nakano 1983). From the present experiment no conclusions can be made concerning the presence of the hydantoin moiety of the molecule in the brain. The origin of side effects affecting the central nervous system such as dizziness, light-headedness, and drowsiness which accompany both acute intravenous and chronic oral administration of D (Harrison 1988), remains unanswered.

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Chapter 11

# PHARMACOKINETICS OF INTRAVENOUSLY ADMINISTERED DANTROLENE AND ITS 5-HYDROXYMETABOLITE IN DOGS

# PHARMACOKINETICS OF INTRAVENOUSLY ADMINISTERED DANTROLENE AND ITS 5-HYDROXY METABOLITE IN DOGS[1]

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**Summary**

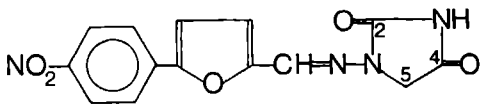
Dantrolene, a direct acting muscle relaxant used orally for spasticity, has appeared to be effective in the prevention and treatment of malignant hyperthermia in man and animals when administered intravenously Its pharmacokinetics following intravenous administration have been studied in dogs. Concentrations of dantrolene and its metabolites in plasma, urine, and bile were determined by high-performance liquid chromatography. Recovery of unchanged drug and reduced metabolites was negligible; of the hydroxy metabolite 2% was found in the urine and about 25% in the bile. The half-life of 5-hydroxydantrolene was shorter than that of the parent drug as demonstrated by administration of the metabolite. The apparent renal clearance of 5-hydroxydantrolene was independent of creatinine clearance, urine flow and pH, and appeared to be reduced in the presence of probenecid. Bile to plasma ratios of the hydroxy metabolite were high with biliary concentrations far exceeding the maximum solubility in water. The results of this pilot study indicate that hydroxylation is primarily responsible for the excretion of the dantrolene molecule from the body.

[1]Int J Clin Pharm Res (in press).

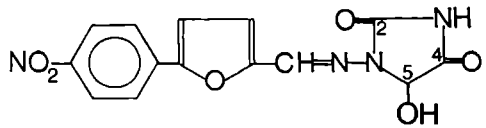
## Introduction

The direct acting muscle relaxant dantrolene (1-{[5-(p-nitrophenyl)-furfurylidene] amino}-imidazolidine-2,4-dione) (Fig. 1) has been used in the management of patients with spasticity orally for more than 20 years. Because it interferes with the release of calcium from the sarcoplasmic reticulum, muscle contraction is impaired. From 1975, intravenous dantrolene has been recommended in man and other species including the dog for the prevention and treatment of malignant hyperthermia, a syndrome which has previously been associated with a high mortality rate (1-4). Dantrolene is metabolized by hydroxylation of the hydantoin ring and by reduction of the nitro group, which in some species is followed by acetylation (5,6). Intermediates of the reduction pathway have been associated with hepatic injury (7). Hydroxylated dantrolene (Fig. 1) has been reported to contribute to the pharmacological response (8,9).

Although its efficacy is now well established, there is little information on the disposition of dantrolene or its metabolites. Renal excretion of unchanged dantrolene was negligible both in a dog and in man (10-12). The hydroxy metabolite and the reduced acetylated compound have been recovered in human urine; in the dog only 5-hydroxydantrolene was found. In man, the mean half-life of dantrolene was reported to be shorter than that of its hydroxy metabolite (13,14), whereas we found in a patient the decay of 5-hydroxydantrolene to parallel parent drug




dantrolene




5-OH-dantrolene

FIGURE 1
Structures of dantrolene and its metabolite 5-hydroxydantrolene

loss (15). The purpose of the present pilot study in the dog was to investigate, using both plasma and urine data, the pharmacokinetics of dantrolene, in particular with respect to its elimination via hydroxylation. Since the urinary mass balance of intravenously administered dantrolene in man and dog is far from complete (11,12), the possibility of biliary excretion - a pathway known to exist in the dog for the structurally related drug nitrofurantoin (16) - was also investigated.

## Materials and methods

### DRUGS AND FORMULATIONS

Dantrolene (sodium. 3 $H_2O$), 5-hydroxydantrolene, aminodantrolene, acetylaminodantrolene, amino-5-hydroxydantrolene, and acetylamino-5-hydroxydantrolene were kindly supplied by Norwich-Eaton (Norwich, USA). The other drugs used were obtained from commercial sources: probenecid (Sigma, St. Louis USA), pentobarbital sodium (Narcovet®, Amerpho, Arnhem, The Netherlands).

Prior to administration, the dantrolene or 5-hydroxydantrolene dose and 4.5 g of mannitol were dissolved in 90 ml of water for injection; the pH was adjusted to 9.5 with sodium hydroxide. To make a loading dose, probenecid was dissolved in 30 ml of normal saline solution; to make a maintenance dose, in 60 ml. The pH of both was adjusted to 9.5 with sodium hydroxide.

### ANIMAL EXPERIMENTS

Three beagle dogs, two females (10.7-11.4 kg body weight) and one male (14.5 kg) were obtained from the Central Animal Laboratory of the University of Nijmegen. The dogs were used several times with a washout period of at least one week between the experiments. At the beginning of each experiment the animals were anaesthetized with pentobarbital sodium (30 mg.kg $^{-1}$). Small additional doses of the anaesthetic were given as needed to keep the dogs asleep for 7 h. Hydration was maintained by continuous intravenous infusion with a solution of 2% mannitol in normal saline. After recovery from anaesthesia, the animals were housed in metabolic cages enabling collection of spontaneously voided urine.

Dantrolene or 5-hydroxydantrolene (2.6-3.9 mg.kg$^{-1}$) was administered by constant rate intravenous infusion for 45 min with a flow rate of 2 ml.min$^{-1}$. When a competitive inhibition experiment on active secretion was carried out, probenecid was co-administered starting with an intravenous loading dose of 15 mg.kg$^{-1}$ in 15 min (flow rate 2 ml.min$^{-1}$) and followed by a maintenance dose of 2.5 mg.kg$^{-1}$.h$^{-1}$ by constant rate infusion (15 ml.h$^{-1}$) to attain steady-state plasma concentrations between 50 and 100 mg.l$^{-1}$ (17). In total eight experiments were carried out with the three dogs.

SAMPLING PROCEDURES

During the anaesthesia period 2-ml blood samples drawn from a central venous catheter were collected in heparinized tubes at predetermined intervals. These samples were immediately centrifuged (1500 g) and plasma was stored at -20°C pending analysis. Urine was collected through a urinary catheter every 30 min. Care was taken that the bladder was empty at the end of each collection period. The total volume and pH of each specimen were measured and 10-ml samples were stored at -20°C pending analysis. In two experiments the animals were also subjected to peri-operative bile drainage. Bile was collected every 30 min and handled as urine. After recovery from anaesthesia, urine collection and blood sampling were continued for a total period of 24 h.

ANALYSIS OF PLASMA, URINE, AND BILE

Dantrolene, 5-hydroxydantrolene, and acetylaminodantrolene in plasma and urine were measured by high performance liquid chromatography (HPLC) as described previously (12). When the water content of the mobile phase was slightly increased, aminodantrolene and the reduced hydroxylated compounds could be measured simultaneously. Bile samples were homogenized by mixing on a vortex, diluted 1:100 with water, and directly injected. Plasma probenecid concentrations were determined by HPLC (18). Creatinine both in plasma and urine was measured throughout each experiment by an automated Jaffé method.

LIGHT PROTECTION

During the entire experiment, from the preparation of the infusion fluids through to the analysis of the samples, care was taken that all solutions and body fluids containing dantrolene or metabolites were kept protected from light by wrapping the syringes, tubing, collection vessels etc. in aluminum foil.

PHARMACOKINETIC AND STATISTICAL ANALYSIS

Elimination half-lives ($t_{\frac{1}{2}}$) were determined by linear regression analysis of the terminal region of the log plasma concentration versus

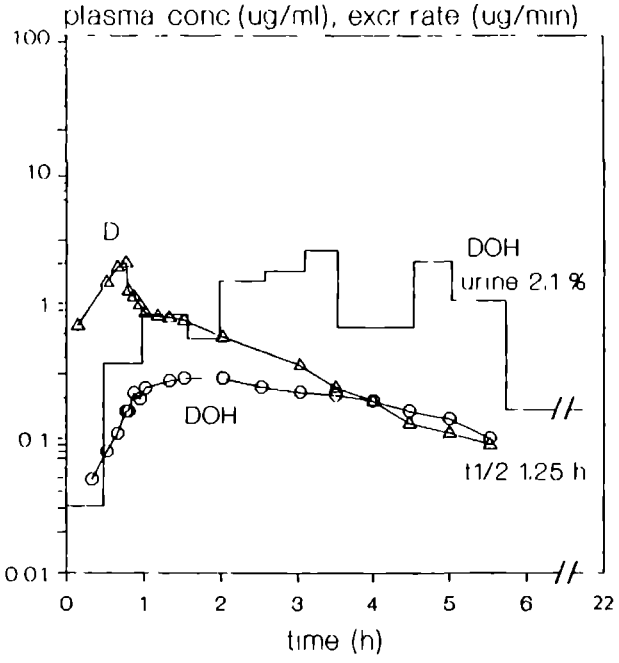



FIGURE 2

Plasma concentration-time curves and renal excretion rate-time profile of dantrolene (D) and its metabolite 5-hydroxydantrolene (DOH) in dog 758 after intravenous administration of 2.8 mg.kg$^{-1}$ of dantrolene sodium. The half-lives of both compounds tended to become similar. The infusion time was 45 min

time curves. Areas under the curve (AUC) were calculated by the linear trapezoidal rule with extrapolation to infinity. The total body clearance (CL) and the apparent volume of distribution ($V_z$) were computed according to CL = D/AUC (D being dose administered), and $V_z = CL.t_{\frac{1}{2}}/0.693$.

An overall value of the apparent renal clearance ($CL_R$) was obtained by $CL_R = A_e^U/AUC$, in which $A_e^U$ is the amount excreted into the urine. An overall value of the biliary clearance ($CL_B$) was similarly obtained from $A_e^B$ (amount excreted into the bile). The mean $CL_R$ and $CL_B$ values with standard deviation were computed using either the renal or the biliary excretion rate in each urine or bile specimen, divided by the plasma concentration at the mid-point of the measured time interval. The mean creatinine clearance values ($CL_{CR}$) were similarly obtained.

Data are expressed as mean ± s.d. Statistical significance was tested by t-tests; P less than 0.05 was taken to indicate significance.

## Results

### DANTROLENE

The plasma concentration-time curves and the renal excretion rate-time profile of dantrolene and its metabolite 5-hydroxydantrolene in one dog after a single intravenous infusion of 2.8 $mg.kg^{-1}$ are shown in Fig. 2. The half-life of the parent drug in this dog was 1.25 h, while the decay of the metabolite tended to parallel parent drug loss. Urinary recovery was low, 2.13% of the dose was excreted as the hydroxy metabolite, while recovery of unchanged drug and reduced metabolites was negligible. Comparable data were found in the other dogs. Individual values of some pharmacokinetic parameters are given in Table Ia. Individual mean values of renal function for each dog as calculated from post-infusion data are listed in Table II. The apparent renal clearance of 5-hydroxydantrolene under the experimental conditions was not clearly related to the creatinine clearance, urine pH, or urine flow. With very low flow rates below 0.2 $ml.min^{-1}$ both the creatinine clearance and the renal clearance of the metabolite were reduced.

In the dog with peri-operative bile drain, no unchanged drug or reduced metabolites were detected in the bile samples, but concentrations

of 5-hydroxydantrolene were high. Metabolite recovery from bile was about 21% of the dose; although the utmost care was taken, some leakage of bile into the duodenum did occur. Individual mean values of biliary function for this dog as calculated from post-infusion data are also listed in Table II. The apparent biliary clearance of 5-hydroxydantrolene under the experimental conditions was independent of bile flow and pH.

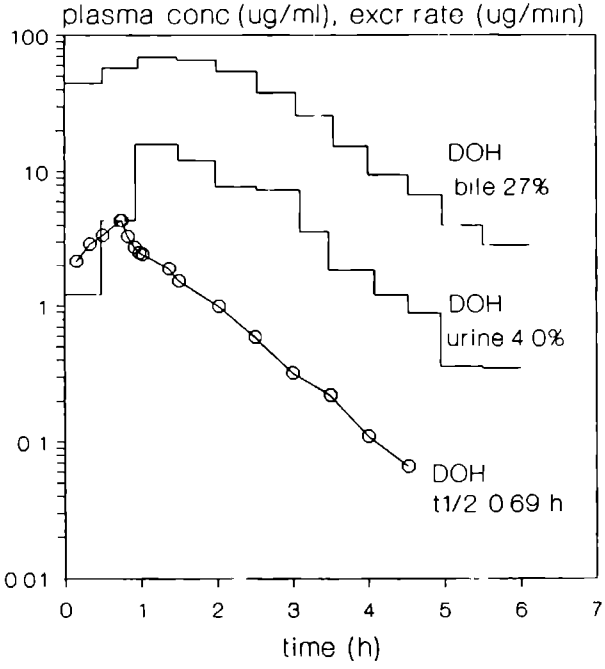



FIGURE 3

Plasma concentration-time curve and renal and biliary excretion rate-time profiles of 5-hydroxydantrolene (DOH) in dog 758 with bile fistula after intravenous administration of 3.7 mg.kg$^{-1}$ of 5-hydroxydantrolene. The infusion time was 45 min

5-HYDROXYDANTROLENE

The pharmacokinetic parameters of 5-hydroxydantrolene when administered as parent drug are listed in Table Ib. The intrinsic half-life of 5-hydroxydantrolene was shorter than that of dantrolene. Its urinary recovery was low; recovery of reduced metabolites was negligible. Individual mean values for the renal function of each dog as calculated from post-infusion data are given in Table II. The $CL_R$ was not clearly related to $CL_{CR}$, urine pH, or urine flow.

Fig. 3 shows the pharmacokinetic profile of 5-hydroxydantrolene in the same dog as depicted in Fig. 2. Biliary recovery of unchanged 5-hydroxydantrolene in this dog was 27%. Individual mean values for the biliary function during the post-infusion period are also given in Table II. The apparent biliary clearance was independent of bile flow and pH.

PROBENECID

Serial plasma concentration measurements of probenecid led to mean levels of over 60 $mg.l^{-1}$ as listed in Table II. After probenecid administration, the apparent renal clearance of 5-hydroxydantrolene decreased; when dantrolene was administered as the parent drug, this decrease reached statistical significance ($p < 0.005$).

## Discussion

The parallel decay of 5-hydroxydantrolene to dantrolene loss suggests that elimination of the hydroxy metabolite is rate-limited by parent drug elimination. That the intrinsic elimination half-life was indeed shorter than that of dantrolene, was demonstrated by metabolite administration. Figure 2 illustrates that too short a sampling time will overestimate the elimination half-life of 5-hydroxydantrolene, when present as metabolite. These findings in the dog can explain the earlier mentioned observations in man, where limited sampling over a relatively short interval led to the supposedly longer half-lives for the metabolite.

TABLE Ia

Pharmacokinetic parameters of dantrolene (D) and its metabolite 5-hydroxydantrolene (DOH) in dogs after intravenous infusion of dantrolene sodium

| Dog No. | Body wt. (kg) | Dose (mg.kg$^{-1}$) | $t_{\frac{1}{2}}$ D (h) | AUC D (mg.h.l$^{-1}$) | $V_z$ D (l.kg$^{-1}$) | %U DOH | %B DOH | CL D | $CL_R$ DOH** | $CL_B$ DOH** |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | (ml.min.kg$^{-1}$) | | |
| 758 | 10.7 | 2.8 | 1.25 | 2.93 | 1.35 | 2.13 | | 12.5 | 0.67 | |
| 576 | 11.4 | 2.6 | 0.78 | 1.55 | 1.49 | 2.22 | | 22.1 | 0.86 | |
| 207* | 14.5 | 2.7 | 1.21 | 4.63 | 0.79 | 4.25 | 21 | 7.52 | 0.65 | 3.2 |

TABLE Ib

Pharmacokinetic parameters of 5-hydroxydantrolene (DOH) in dogs after intravenous administration of 5-hydroxydantrolene as parent drug

| Dog No. | Body wt. (kg) | Dose (mg.kg$^{-1}$) | $t_{\frac{1}{2}}$ DOH (h) | AUC DOH (mg.h.l$^{-1}$) | $V_z$ DOH (l.kg$^{-1}$) | %U DOH | %B DOH | CL DOH | $CL_R$ DOH** | $CL_B$ DOH** |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | (ml.min.kg$^{-1}$) | | |
| 758 | 11.3 | 2.7 | 0.73 | 2.62 | 1.08 | 1.43 | | 17.1 | 0.25 | |
| 576 | 11.3 | 2.6 | 0.50 | 1.98 | 0.95 | 1.85 | | 21.9 | 0.40 | |
| 758* | 11.3 | 3.9 | 0.69 | 5.62 | 0.69 | 4.04 | 27 | 11.5 | 0.46 | 3.1 |

% DOH = percentage of the dose excreted into the urine (U) or bile (B) as 5-hydroxydantrolene.
*with bile drain.
**calculated from $A_\infty$(U or B)/AUC.

The data from the present study indicate that the smaller apparent volume of distribution of the hydroxy metabolite may have caused its shorter half-life. The role of the total body clearance is less clear. Individual components of this composite parameter will be discussed separately. However, from the overall values of the clearances listed in Table I, it can be seen that only a minor fraction of the individual components is known at present.

The apparent renal clearance of dantrolene was found to be negligible. The apparent renal clearance of 5-hydroxydantrolene as calculated from post-infusion data was at least twice as low as the creatinine clearance and was not clearly correlated to the parameters for passive tubular reabsorption. The unbound renal clearances could not be calculated because no data on the extent of binding of dantrolene (or 5-hydroxydantrolene) to plasma protein are available. Although dantrolene is known to have a high affinity for albumin (19), a property shared with the related anionic nitrofurans (20), the bound percentage has not yet been established. Ultrafiltration in the present study failed because of nonspecific binding of unbound drug or metabolite to the filters. Binding experiments are further complicated by aggregation of unbound to bound drug as put forward by Capomacchia and coworkers (21). Taking this into account, the unbound renal clearances might be higher, even indicating active tubular secretion as demonstrated in the dog for nitrofurantoin (22). For 5-hydroxydantrolene, the experiments with probenecid seem to support this hypothesis, but the relevance of this finding for the dog is limited, since renal excretion of 5-hydroxydantrolene is only a minor pathway of elimination. In man, however, where a higher percentage of the dose has been recovered in the urine as hydroxy metabolite (11), the possibility of active renal tubular transport warrants further investigation.

In agreement with a previous dog experiment (12), it was found that when only urine is taken into account about 98% of the mass balance is missing. The absence of the acetylated compound is in agreement with the inability of the dog to acetylate aromatic amino groups (23). Its precursor aminodantrolene, however, was also not detected, nor was any product of the combined metabolic pathways. Further degradation under the influence of light (12) was ruled out as all the relevant material

TABLE II

Renal (R) and biliary (B) handling of 5-hydroxydantrolene (DOH) in dogs after dantrolene and 5-hydroxydantrolene administration (calculated from post-infusion data)

| Dog | $CL_R$ DOH** | $CL_{CR}$ | Urine flow | Urine pH | $CL_B$ DOH** | Bile flow | Bile pH | $C_{prob}$ |
|---|---|---|---|---|---|---|---|---|
| No. | (ml.min.kg$^{-1}$) | | (ml.min$^{-1}$) | | (ml.min.kg$^{-1}$) | (ml.min$^{-1}$) | | (mg.l$^{-1}$) |
| Dantrolene | | | | | | | | |
| 758 | 0.78±0.36 | 2.61±0.99 | 0.46±0.16 | 8.0±0.6 | | | | |
| 576 | 1.24±0.69 | 2.76±0.96 | 0.65±0.28 | 8.2±0.5 | | | | |
| 207* | 0.85±0.24 | 2.38±0.27 | 0.53±0.47 | 8.5±0.1 | 7.52±1.52 | 0.17±0.09 | 8.5±0.4 | |
| 576 | 0.23±0.06 | 3.22±0.44 | 0.33±0.15 | 7.6±0.9 | | | | 67±4 |
| + probenecid | | | | | | | | |
| 5-Hydroxydantrolene | | | | | | | | |
| 758 | 0.48±0.15 | 3.04±0.67 | 0.21±0.05 | 6.9±0.2 | | | | |
| 576 | 0.78±0.52 | 3.24±1.78 | 0.43±0.26 | 5.9±0.2 | | | | |
| 758* | 1.15±0.28 | 2.77±0.65 | 0.35±0.20 | 8.0±0.1 | 7.88±1.85 | 0.12±0.04 | 8.3±0.1 | |
| 576 | 0.42±0.07 | 3.27±0.81 | 0.35±0.21 | 6.2±0.9 | | | | 63±1 |
| + probenecid | | | | | | | | |

$C_{prob}$ = probenecid plasma concentration.

*with bile drain.

**calculated from excretion rate/plasma concentration.

was protected from light as described above. This implies that other pathways must exist for the elimination of dantrolene from the canine body.

Excretion of drugs and their metabolites into bile is a possibility for compounds with a relative molecular mass of over 300 who possess one or more polar groups (24,25). Unchanged dantrolene (mol. mass 314) with its ionisable NH group ($pK_a$ 7 5, Data sheet, Norwich Faton Pharmaceuticals) was not excreted into dog bile in quantity. That the introduction of a hydroxyl group as in the case of 5-hydroxydantrolene dramatically enhanced the extent of biliary excretion, has been described earlier for other drugs (24). Rather than an effect on molecular size or polarity, it has been proposed that the altered chemical structure favours biliary excretion. The high bile to plasma concentration ratios that we found for 5-hydroxydantrolene (100-1000) suggest active transport as established for nitrofurantoin in the dog (16). Since animal viability upon repeated biliary cannulation is poor, no data on competitive binding experiments with probenecid, which can interfere with both renal and biliary transport (25), are as yet provided. Considering its poor water solubility (< 10 $mg.l^{-1}$, Data sheet, Norwich Faton Pharmaceuticals), entrapment by micelles is another likely explanation for the high biliary concentrations of 5-hydroxydantrolene, which could reach values of over 200 $mg.l^{-1}$ (26). Passive transport would be relatively unimportant as the apparent biliary clearance of this metabolite was not dependant upon bile flow and pH in the range investigated.

The difference in the overall values for the biliary clearance (Table I) and the values obtained during elimination (Table II) is partly explained by the existence of a lag time (Fig. 3), which was not corrected in the data for Table II. A lag time may also explain some of the differences found in the earlier mentioned renal clearance values. As biliary excretion can be species dependent and the dog is considered to be a good biliary excreter (24), the results as such cannot be transferred to man or other species. In man, negligible quantities of drug and metabolite have been discovered in preliminary experiments where bile was sampled from post-cholecystectomy patients, when dantrolene was given either orally (27) or intravenously (unpublished observation).

In conclusion, the results of the present study indicate that hydroxylation is an important metabolic pathway for dantrolene in the dog, providing a compound with a shorter intrinsic half-life that can be excreted from the body, apparently predominantly via the bile. As about 70% of the mass balance is still missing following parent or metabolite administration, the need of further kinetic studies on metabolites such as recently was initiated for azomethine cleavage is obvious (28).

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# DANTROLENE PLASMA AND URINE CONCENTRATIONS AFTER ORAL PRETREATMENT FOR MALIGNANT HYPERTHERMIA: REPORT OF A CASE

# DANTROLENE PLASMA AND URINE CONCENTRATIONS AFTER ORAL PRETREATMENT FOR MALIGNANT HYPERTHERMIA: REPORT OF A CASE[1]

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**Summary**

Dantrolene plasma concentrations and renal clearance calculations in a patient undergoing major surgery are reported. The family history was positive for malignant hyperthermia. Following oral pretreatment the plasma concentrations in this patient were not noticeably different from values obtained in a study in awake volunteers and which were thought to be effective. This is the first report of dantrolene measurements made during anaesthesia and in the post-operative period

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Although there is general agreement on the use of intravenous dantrolene during malignant hyperthermia (MH), the mode of administration for prophylaxis is still controversial. When administered orally 1 or 2 days pre-operatively, dantrolene prophylaxis (1-2 mg kg $^{-1}$ four times a day) has been successful in most cases (1-3). Very recently, both oral and intravenous pretreatment failed to protect a 7-year old boy against an episode of MH (4). Dantrolene plasma concentrations were not measured in any of these cases.

It has been suggested by results obtained in a study using normal awake volunteers that blood concentrations of 2.4 mg litre $^{-1}$ could be effective (5). Dantrolene blood concentrations were measured in a patient undergoing major surgery to determine whether concentrations of 2.4 mg litre $^{-1}$ can be achieved following oral administration.

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[1]Eur J Anaesthesiol 1986; 3: 219-23.

## Case report

A 60-year-old woman (66.5 kg) was admitted for biopsy of the breast. Her family history was positive for MH: two cousins had died presumably because of MH. Dantrolene was given orally in the 28 h prior to surgery. Dantrolene sodium 50 mg was given orally at 08.00, 75 mg at 12.00, 75 mg at 16.00, and 75 mg at 20.00; an additional dose of 75 mg was administered orally at 07.00 on the day of surgery. The total dantrolene dose was 350 mg (5.3 mg $kg^{-1}$).

Pre-anaesthetic sedation consisted of 10 mg diazepam orally, 5 mg droperidol i.m., and 7 mg nicomorphine i.m. Anaesthesia was induced with 0.15 mg fentanyl and 450 mg thiopentone i.v., and maintained with nitrous oxide 70% in oxygen and intermittent fentanyl (total 0.4 mg i.v.). An i.v. bolus injection of 45 µg $kg^{-1}$ vecuronium was given to facilitate endotracheal intubation. A mastectomy was performed following a positive biopsy. The course of anaesthesia (4.5 h) and the postoperative period were uneventful.

## Method

Blood samples were drawn hourly during anaesthesia and at intervals of 3-5 h post-operatively over a total period of 50 h. Urine that had been voided spontaneously was collected during the same period. A reversed phase high-performance liquid chromatographic method was used to determine the concentrations of dantrolene and its metabolites in plasma and urine (6). Creatinine concentration was also measured in all plasma and urine samples. The renal excretion rates and the renal clearances were calculated using standard methods (7). Plasma half-lives were determined by linear regression of the terminal parts of the log plasma concentration versus time plots. Peroperatively a muscle biopsy was taken. Susceptibility for MH was tested according to the protocol of the European Malignant Hyperpyrexia Group (8).

Following oral pretreatment dantrolene plasma concentrations were between 1.7 and 3.6 mg litre $^{-1}$ during anaesthesia (Fig. 1). Post-operatively the concentrations declined with a half life of 15 h. For the main metabolite, 5-hydroxydantrolene, the same half-life was found. The

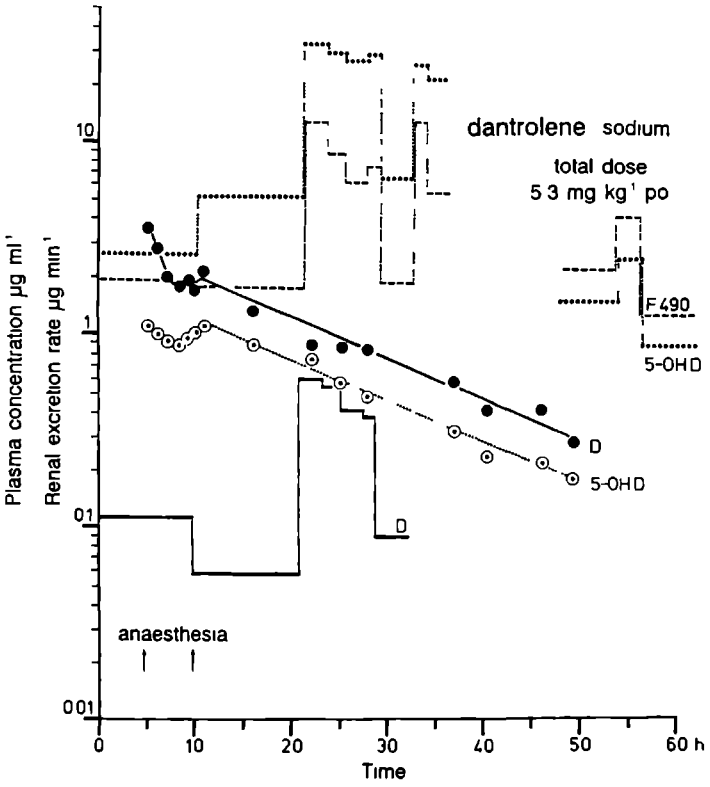


FIGURE 1

Plasma concentration-time and renal excretion rate-time profiles of dantrolene (D) and its metabolites: 5-hydroxydantrolene (5-OH D) and nitro-reduced acetylated dantrolene (F 490), in a patient pretreated with divided oral doses of dantrolene sodium. The last oral dose (75 mg) was given at t = 0. Each horizontal bar represents a urine collection interval

renal excretion rates of dantrolene and its metabolites were markedly depressed on the day of surgery and this led to low renal clearance values: 0.04 ml min $^{-1}$ and 0.4 ml min $^{-1}$ for dantrolene and 5-hydroxy dantrolene, respectively. Post-operatively renal clearances were approximately ten times higher in both cases.

On the day of surgery the creatinine clearance was 45 ml min $^{-1}$ and the urinary flow rate 0.4 ml min $^{-1}$ (Fig. 2). Post-operatively, a creatinine clearance of 130 ml ml $^{-1}$ was calculated; the urinary flow rate was approximately 3 ml min $^{-1}$.

## Discussion

The prophylactic dose regimen of dantrolene, for MH, seems to be related to the pharmacokinetics of the drug. Blood levels which were achieved with the recommended oral dose regimens in patients who were undergoing surgery have not previously been reported (5). Following studies in volunteers and in spastic patients, the use of dantrolene i.v. has been encouraged because the blood levels were more predictable than those recorded following oral doses (5). Dantrolene concentrations in a patient undergoing surgery and who was orally pretreated are presented in this case report. Plasma concentrations are reported here but the data are not directly comparable with that presented by Flewellen and co-workers in 1983 (5) and who reported whole-blood levels. In humans whole-blood concentrations have been found to be approximately 1.4 times greater than the concentrations in plasma (unpublished observations). If this is taken into account, oral pretreatment led, in the case reported here, to accepted effective dantrolene concentrations. The half life was also within the range previously reported (5). The importance of the dantrolene prophylaxis in this patient remains unknown. No 'triggering' anaesthetics were given and the responses of the muscle biopsy were negative. The latter finding should be interpreted with caution as the biopsy was taken after dantrolene pretreatment (9).

Anaesthesia and surgery may influence the renal excretion of several compounds and therefore the urine, drug concentrations and renal function were measured. The low creatinine clearance and the low urine flow, on the day of surgery, may have contributed to the markedly re-

duced renal clearance of dantrolene and its chief metabolite 5-hydroxy-dantrolene, when compared with the values found in a group of six normal volunteers. The mean values for the volunteers were 0.2 ml min $^{-1}$ and 67 ml min $^{-1}$, respectively (10).

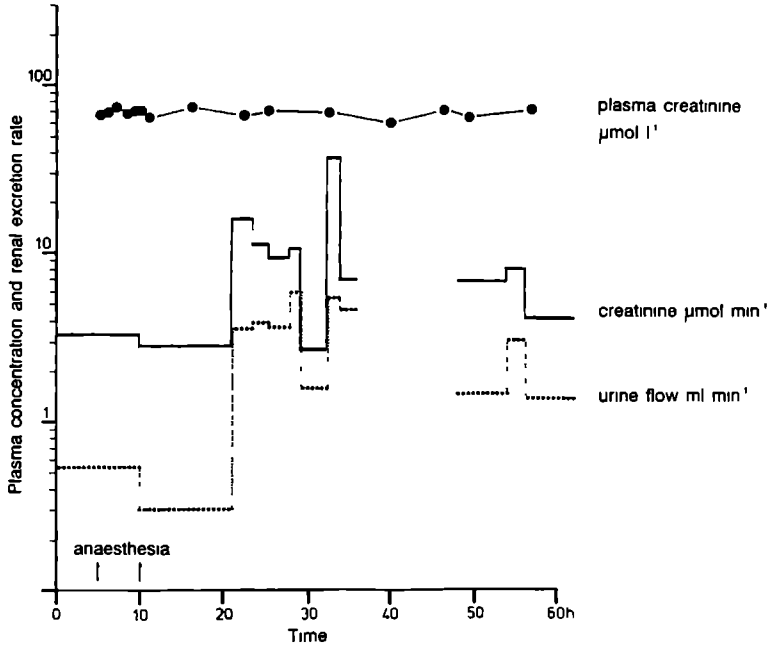


FIGURE 2
Plasma concentration-time an renal excretion rate-time profiles of creatinine, and urine flow-time measurements in a patient during anaesthesia and in the post-operative period. Each horizontal bar represents a urine collection interval.

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# SECTION V

# PHARMACOKINETICS AND SPASTICITY

Chapter 13

# EFFECTS AND PLASMA CONCENTRATIONS OF BACLOFEN AND DANTROLENE IN MENTALLY RETARDED PATIENTS WITH CEREBRAL PALSY: A PRELIMINARY REPORT

# EFFECTS AND PLASMA CONCENTRATIONS OF BACLOFEN AND DANTROLENE IN MENTALLY RETARDED PATIENTS WITH CEREBRAL PALSY: A PRELIMINARY REPORT[1]

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## Abstract

Mentally retarded patients with cerebral palsy are often treated with antispastic medication without adequate control of the desired outcome. We report on a group of patients that was suspected of having no (optimal) clinical benefit. The initial treatment policy for the 12 patients reported here, was to withdraw their medication (baclofen in 8, and dantrolene in 4) while monitoring plasma concentration and effect. Plasma concentrations were measured by high-performance liquid chromatography. Effect was tracked by neurological assessments, and ADL performance supported by video recordings. In most cases, minor or inconsistent changes in ADL performance were found across the different treatment phases. In the neurological examination, most patients with major spastic involvement showed a deterioration in overall posture and movement rating. These patients had initial plasma concentrations between 0.12 and 0.24 mg.l $^{-1}$ of racemic-baclofen, between 0.33 and 0.44 mg.l $^{-1}$ of dantrolene. The findings of this pilot study suggest that such levels may be effective in modifying abnormal motor manifestations; no relation to functional response in the everyday-life situation was yet found.

[1]Arch Phys Med Rehabil (submitted)

**Key words:** Antispastic medication; Baclofen; Cerebral palsy; Dantrolene; Effects; Mental retardation; Plasma concentrations

Cerebral palsy is a non-progressive disorder of movement and posture due to a defect or lesion of the immature brain (1). The lack of motor control in patients with this chronic ailment typically presents as spasticity, involuntary movements, and/or incoordination. Most commonly, spasticity is the dominant manifestation. In addition to the motor deficit, other major disabilities of cerebral palsy are mental retardation in 50% and seizure disorders in 25% of the patients (2). Medications for patients with spastic cerebral palsy often include baclofen and/or dantrolene (3,4), while dantrolene has also been given for control of involuntary movements (5,6). Assessment of the outcome of a given therapy is, however, still a major problem. A variety of methods has been developed to assist in therapeutic decisions for these patients (3,6-9), but most are not feasible for severe mental retardation.

In our institution for the mentally retarded, 100 patients had disorders of motor control associated with cerebral palsy, a third of whom were on long-term antispastic medication. From incidental reports and observations we concluded that many patients were suspected of having no (optimal) clinical benefit. We have used simple tests and plasma concentration measurements to investigate response to antispastic treatment in this particular patient population. Drug plasma concentrations were included in this pilot study, since this approach has previously proved to be successful for regulating drug therapy for epilepsy and other chronic disorders with a hard to evaluate therapeutic effect. All patients received a baseline neurological examination to document their present status and to set individual treatment goals. We report on the outcome for 12 patients who under went a reduction of their medication (baclofen in 8 patients, dantrolene in 4) as the initial treatment policy.

## Methods

### PATIENTS

Table I summarises the characteristics of each of the 12 patients (11 females and 1 male). The female domination reflects a previous admission policy of our institution. Depending upon the principal handicap, a patient was classified either as spastic or dyskinetic, the latter presenting as choreo-athetosis, rigidity, dystonia, and/or tremor The relatively low body weights are normal for these patients (10). Each patient had at least mild mental impairment. Vision and speech deficits

TABLE 1

Patient Characteristics

| Patient* -Motor† Disorder | Age/Weight (yr/kg) | Mental retardation | Vision | Speech | Locomotion |
|---|---|---|---|---|---|
| 1-SP | 46/39 | severe | good | no | walks self |
| 2-SP | 37/49 | severe | good | no | walks with help |
| 3-SP | 43/48 | severe | good | no | walks with help |
| 4-SP | 59/58 | moderate | good | yes | walks with help |
| 5-SP | 29/41 | profound | diminished | no | bed-ridden |
| 6-SP | 44/40 | severe | good | no | bed-ridden |
| 7-DYS | 32/40 | severe | good | yes | chair-bound |
| 8-DYS | 44/44 | moderate | good | no | chair-bound |
| 9-SP | 21/48 | mild | diminished | yes | walks with help |
| 10-SP | 12/38 | mild | diminished | yes | chair-bound |
| 11-SP | 13/22 | profound | good | no | chair-bound |
| 12-SP | 16/32 | profound | blind | no | bed-ridden |

*All patients are female, except patient 5.

†SP denotes spasticity is major handicap, and DYS dyskinetic involvement.

were common. All patients were on long-term baclofen and/or dantrolene treatment for 5 years or longer. Physiotherapy and drug therapy for epilepsy and other disorders were continued as needed. Only 1 patient received a benzodiazepine drug.

DRUGS AND PLASMA CONCENTRATIONS

Baclofen was given as 10-mg tablets (Lioresal®, Ciba-Geigy), which contain baclofen in its racemic form. Dantrolene sodium was given as a 5-mg ml $^{-1}$ suspension (Dantrium®, Norwich Eaton); patients treated previously with 25-mg capsules were changed to identical doses of the sus-

TABLE I
(continued)

| Orthopaedic surgery | Physio-therapy | Drug ‡ therapy | Co-medication ‖ |
|---|---|---|---|
| no | no | B | AED, laxatives |
| hip, spinal cord | no | B | AED, laxatives |
| tendons | no | B | AED, hormone, laxative, psychoactive drug |
| hip, tendons | no | B | AED, diuretic, laxatives |
| no | yes | B | AED, laxatives, vitamin |
| tendons | yes | B | AED, hormone, laxatives |
| tendons | yes | B,d | hormones |
| hip | yes | B | anticholinergics, laxatives |
| tendons | yes | D,B | laxative |
| tendons | yes | D,B | AED |
| tendons | yes | D,B | |
| tendons | yes | D,B | AED, laxatives, mucolytic |

‡B denotes baclofen, D dantrolene, and d diazepam.

‖AED denotes antiepileptic drugs.

pension. Generally, doses were given 3-times daily. Baclofen was withdrawn at a rate of 5 mg a week as recommended by Terrence and Fromm for patients on long-term treatment (11). Dantrolene dosage was reduced at a rate of 15 mg a week.

Plasma concentrations were measured initially, and when feasible during reduction of the medication. Blood was drawn approximately 3 hours after the morning dose; plasma was stored at -20°C pending analysis. Dantrolene and its metabolite 5-hydroxydantrolene and rac baclofen were determined by high-performance liquid chromatography as previously described (12,13). The total body clearance (CL/f) was calculated from the dose per dosage interval divided by the corresponding plasma concentration.

TESTING OF PATIENTS

Patients were tested initially, during medication reduction when feasible, and after drug withdrawal. Testing consisted of 3 assessments: (1) clinically grading of muscle tone and of posture and movements status by a (paediatric) neurologist; (2) performance of daily living activities (ADL) by an attendant charged with patient supervision; and (3) evaluation by 3 observers (consultant physicians) of motor performance recorded by video.

Muscle tone was graded using a 5-point scale: -2 = considerable decrease in tone; -1 = mild decrease in tone; 0 = no increase or decrease in tone; +1 = mild increase in tone; and +2 = considerable increase in tone. The score was the sum of the score for the neck, trunk, arms, hands, legs, and feet divided by 6. When the tone of the extremities was not equal on both sides, the average of the scores for the two sides was taken. Posture and movements were graded on a 3-point scale. Four check lists with 6 items each (neck, trunk, arms, hands, legs, and feet) covered posture, spontaneous movement as impaired by abnormal tone, spontaneous movement as impaired by abnormal patterns, and controlled movement towards desired object. Left-right differences were handled as described above. The percentage of the total score for all 24 items constituted the measure. The locomotive potential determined whether a patient was examined supine, seated, or standing upright.

The ADL assessments were adapted to the degree of the patient's disability, and of necessity these were individualised. Several different behaviours with regard to motor functioning were included in a check list, and graded using a 3 point scale as recommended by Banham (14). The percentage of the total score for all the behaviours performed constituted the measure. The attendant most familiar with a particular patient participated in drawing up a personal check list, which typically consisted of 4-8 behaviours related to locomotion (standing, walking indoors and outdoors, with aids if needed), arm and hand function (the ability to reach for and hold objects, wheelchair use), and/or ease of management (while getting dressed, etc.). The same attendant was charged with the daily filling in of the check list for that patient for discrete treatment periods. Due to the organisation of the institute, as will be found in other institutes, it could not be avoided that this attendant was also acquainted with the patient's medication. The results were analysed by visual inspection (15). The bed-ridden patients and patient 11, who had almost no voluntary control, followed a slightly different schedule. Their ease of management or range of passive motion in the limbs was graded on a 5-point scale, respectively by an attendant or physiotherapist.

Selected aspects of the motor behaviours mentioned above were recorded on video-tape when pertinent or applicable. Each behaviour was graded separately on a 6-point scale by three observers; their scores were averaged. The mean score for the 5-8 behaviours performed by each patient constituted the measure. During a particular period, each patient was recorded only once. The recordings of the different periods of a patient were viewed in random order and evaluated in a 'blind' manner, i.e. the consultant physicians were unaware which period was being shown. The results were analysed on an individual basis; twice the standard error of the mean had to be exceeded before the qualification improved (or deteriorated) was given. In addition, each of the observers was asked to order the recordings from best to least. No video recordings were made of those patients with almost no voluntary control (see above).

For all scores, 0 represented the best performance.

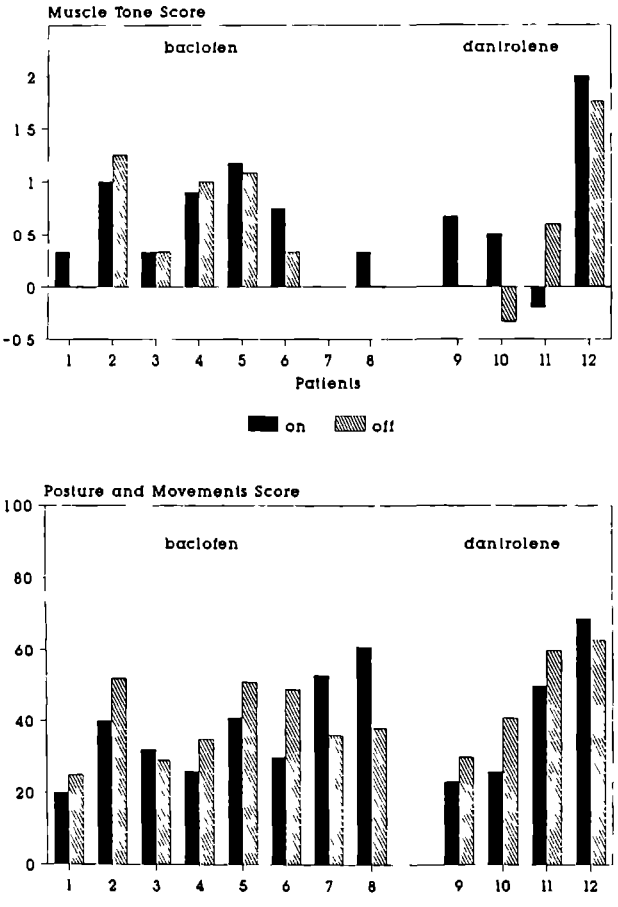


FIGURE 1

Muscle tone and Posture and movements scores with and without baclofen (patients 1-8) or dantrolene (patients 9-12). The black bars represent the score on the initial dose. A thick line on the horizontal axis represents a score of 0

OVERALL NEUROLOGICAL EVALUATIONS

The score for the muscle tone and the posture and movements score of each patient with and without drug are shown in figure 1. For the 8 patients on baclofen (patients 1-8), no significant difference was observed on each evaluation scale between the period of initial medication and that of no drug (Wilcoxon signed rank test). When only the 6 spastic patients on baclofen (patients 1-6) are considered, a significant deterioration in the posture and movements score was found; the mean ($\pm$SD) score increased from 31 ($\pm$8) to 40 ($\pm$12) ($P < 0.05$). In the same group of 6, the 2 periods could not be distinguished based on muscle tone. For the 4 patients on dantrolene (patients 9-12), the results followed a similar trend; the number of patients was insufficient for a statistical test.

Table II lists the initial baclofen and dantrolene plasma concentrations and corresponding total body clearances for each patient.

INDIVIDUAL ADL AND VIDEO EVALUATIONS - CASE REPORTS

- Patient 1, a 46-year-old severely retarded woman with no verbal communication had spastic quadriplegia of unknown etiology. She had developed flexion contractures, but was independently ambulant. For the past 7 years she had been treated with baclofen, 45 mg daily (plasma concentration 0.24 mg.l $^{-1}$). Her epilepsy was held under control with phenytoin 225 mg daily (18 mg.l$^{-1}$), and phenobarbitone, 100 mg daily (23 mg.l$^{-1}$). She also received a bulk laxative and lactulose. The major aim for this patient was to maintain her present walking ability. As shown in figure 2, the mean ADL score for the drug-free period was close to the initial values; on the 20-mg dose, a small deterioration in the mean score was found. On the video scale, gait was slightly better on low or no drug, but failed to get the qualification improved; all three observers ranked the video-tape representing the period of initial dosage as being least. Both evaluation methods agreed that her locomotive behaviour was not better while on the drug. The low plasma concentration (0.04 mg.l $^{-1}$) and high clearance value (347 ml.min $^{-1}$) found for the 20-mg dose suggest a deviation from the agreed sampling time and/or dosage schedule. Since the onset of baclofen withdrawal her

epilepsy has deteriorated. To what extent a temporary reduction in phenytoin dose and level has contributed to this deterioration is not clear. The phenobarbitone plasma concentration had not changed.

Patient 2, a 37-year-old severely retarded woman with no verbal communication had spastic quadriplegia resulting from phenylketonuria. Clear dyskinetic involvement was apparent in arms and hands (choreoathetosis). She had developed fixed deformities, but could walk independently with a little support. For the past 12 years she had been treated with baclofen, 45 mg daily (0.15 mg.l $^{-1}$). Her epilepsy was held

TABLE II
Initial Medications

| Patient | Daily dose (mg) | (mg.kg $^{-1}$) | Plasma concentration (mg.l$^{-1}$) | Total body clearance (ml.min $^{-1}$) |
|---|---|---|---|---|
| Baclofen | | | | |
| 1 | 45 | 1.2 | 0.24 | 130 |
| 2 | 45 | 0.9 | 0.15 | 208 |
| 3 | 30 | 0.6 | 0.14 | 149 |
| 4 | 30 | 0.5 | 0.19 | 110 |
| 5 | 30 | 0.7 | 0.12 | 174 |
| 6 | 45 | 1.1 | 0.22 | 142 |
| 7 | 45 | 1.1 | 0.22 | 142 |
| 8 | 30 | 0.7 | 0.15 | 139 |
| mean (±SD) | | | | 149 (±30) |
| Dantrolene | | | | |
| 9 | 75 | 1.6 | 0.44 | 93 |
| 10 | 75 | 2.0 | 0.33 | 124 |
| 11 | 75 | 3.4 | 0.42 | 98 |
| 12 | 90 | 2.8 | 0.43 | 115 |
| mean (±SD) | | | | 107 (±15) |

under control with valproate 900 mg daily (44 mg.l $^{-1}$). She also received bisacodyl and lactulose. The major aim was to maintain her present walking ability. As depicted in figure 2, for the period of reduced dosage both evaluation methods showed a deterioration in the score. After the drug had been totally withdrawn, the deterioration was less severe (ADL) or absent (video). Video rank order agreed with the ADL findings. Although both evaluation methods showed a similar trend, the benefit of baclofen for this patient's locomotive behaviour remained unclear. It is noteworthy, that the attendants were of the opinion that walking had become much more difficult since the drug usage was terminated and that it remained poor in the weeks that followed. As was seen for patient 1, the plasma concentration measured during medication reduction while she received 25 mg daily was relatively low (0.06 mg. l$^{-1}$), the clearance high (289 ml.min $^{-1}$). Also, during baclofen withdrawal 2 major seizures occurred in this patient who had been free of major seizures for years. The valproate plasma concentration had not changed.

- Patient 3, a 43-year-old severely retarded woman with no verbal communication had spastic quadriplegia of unknown etiology. Clear dyskinetic involvement was apparent in left arm (athetosis) and left hand (tremor). She had developed minor contractures and could walk when taken by the right hand. For the past 10 years she had been treated with baclofen, 30 mg daily (0.14 mg.l $^{-1}$). Her epilepsy was held under control with phenobarbitone 120 mg daily (30 mg.l $^{-1}$). She also received haloperidol, lynoestrenol, and bisacodyl. The major aim was to maintain her present walking ability. As shown in figure 2, on the ADL scale the mean score for the drug-free period was close to the initial mean value. On the video scale, the period of no drug could be distinguished from that of initial medication by a deterioration in the score, but the difference was small; observer rank order followed that score. Whether baclofen was of benefit for her locomotive behaviour remained unclear. A remarkable improvement of mood coincided with baclofen withdrawal that might have affected the ADL rating. In the opinion of the attendants trembling of the left hand had decreased, which agreed with the findings of the neurological examination. On the 10-mg dose, the

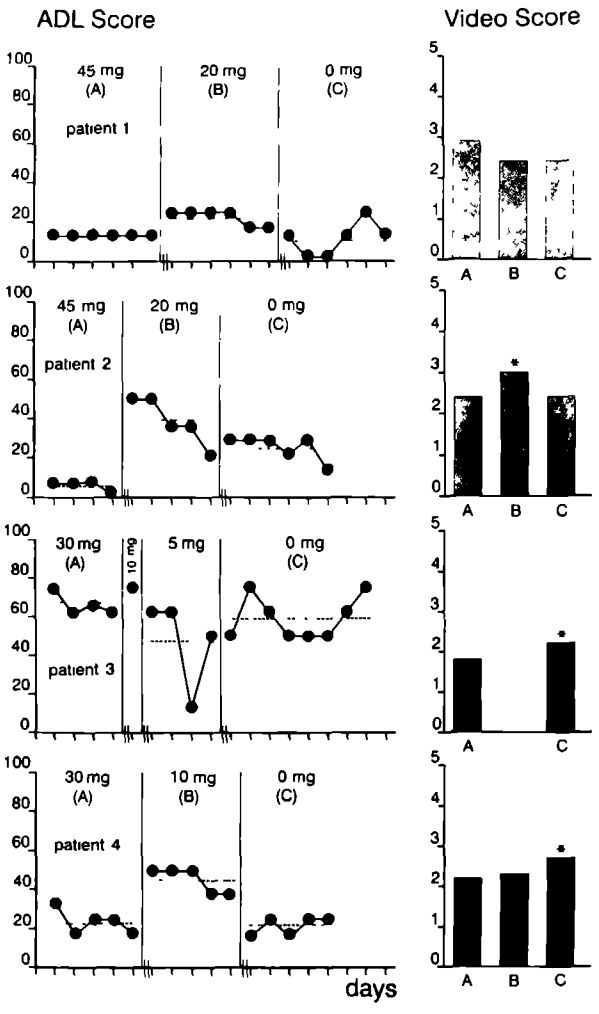


FIGURE 2

ADL (line graphs) and video (histograms) scores for 4 ambulant spastic patients (1-4) during baclofen reduction. In each case, the walking ability was tested. The mean values for the ADL score in each phase are represented by the dashed lines.

*qualified as deteriorated compared to the other video data reported for that patient

plasma concentration was high (0.12 mg.l$^{-1}$), the clearance low (58 ml. min$^{-1}$). Control of her epilepsy had not deteriorated.

- Patient 4, a 59-year-old moderately retarded woman with little verbal communication had spastic quadriplegia of unknown etiology. Slight dyskinetic involvement was apparent in neck and arms. She could walk independently with a little support. For the past 6 years she had been treated with baclofen, 30 mg daily (0.19 mg.l $^{-1}$). Her epilepsy was acceptable with phenytoin, 200 mg daily (6 mg.l $^{-1}$). For mild hypertension she was treated with chlorthalidone. She also received lactulose and bisacodyl. The major aim was to maintain her present walking ability. As shown in figure 2, the mean ADL score for the drug free period was similar to the initial mean value; on the 10-mg dose, a deterioration in the mean score was found. On the video scale, the period of no drug could be distinguished from the two periods while on different doses by a deterioration in the score. For two of the three observers, the rank order followed the video score. Since the assessments disagreed, the benefit of baclofen for this patient's locomotive behaviour remains unclear. It is of note, that the attendants were of the opinion that walking had become more difficult since the drug usage was terminated. Her epilepsy remained difficult to control; the phenytoin dose was increased.

- Patient 5, a 29-year-old profoundly retarded man with no verbal communication and poor vision had spastic quadriplegia resulting from preterm birth asphyxia. He was bed-ridden with almost no voluntary movement or control of posture and had fixed deformities. For the past 9 years he had been treated with baclofen, 30 mg daily (0.12 mg.l $^{-1}$). His epilepsy was held under control with phenobarbitone 100 mg daily (44 mg.l $^{-1}$), valproate 1800 mg daily (67 mg.l$^{-1}$), and carbamazepine 600 mg daily (4.5 mg.l$^{-1}$). He also received ascorbic acid, bisacodyl, and lactulose. The aim was to maintain the present range of passive motion in his arms and legs. Reducing the dosage to no drug at all did not induce any change in the score for arms and legs as recorded by the physiotherapist. On a daily dose of 15 mg, a plasma concentration of 0.09 mg. l$^{-1}$ was measured; the clearance was 116 ml.min$^{-1}$. The findings suggest that this patient did not benefit from baclofen in the doses tested.

Attendant opinion was that nursing care had become just a little more difficult, but that on the whole he was not worse than before. Control of his epilepsy had not deteriorated.

- Patient 6, a 44 year old severely retarded woman with no verbal communication had spastic quadriplegia resulting from perinatal cranial trauma. She was bed ridden with almost no voluntary movement or control of posture and had fixed deformities. For the past 6 years she had been treated with baclofen, 45 mg daily (0.22 mg.l $^{-1}$). Her epilepsy was held under control with phenobarbitone 100 mg daily (25 mg.l $^{-1}$). She also

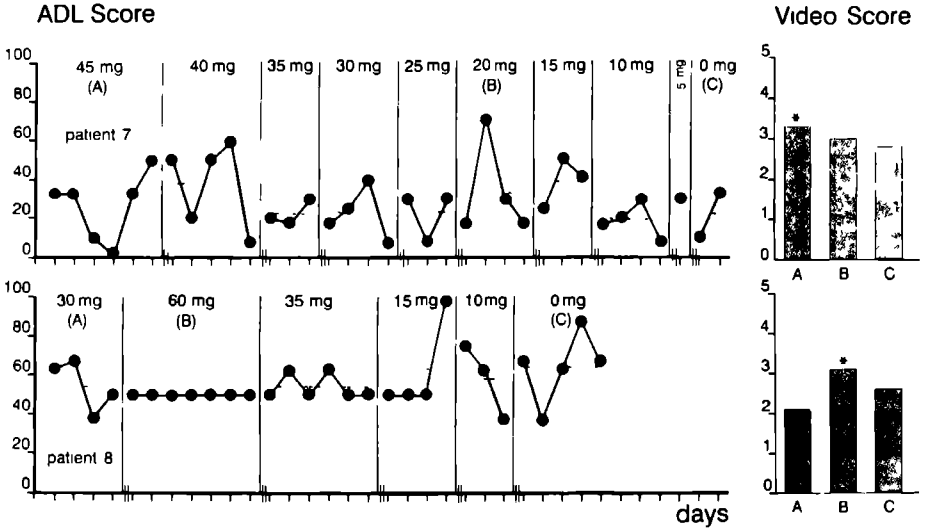


FIGURE 3

ADL (line graphs) and video (histograms) scores for 2 dyskinetic patients (7 and 8) during baclofen dosage alteration. In each case, the tests included arm function and ease of management. Dashed lines as in figure 2.

*qualified as deteriorated compared to C (patient 7) or A and C (patient 8)

received ascorbic acid, bisacodyl, lactulose, and lynoestrenol. The aim was to maintain the present range of passive motion in her legs and arms. As reported for patient 5, for the entire period no change in the score was recorded by the physiotherapist. While treated with 30 mg baclofen daily, the plasma concentration was 0.18 mg.l $^{-1}$, the clearance 116 ml.min $^{-1}$. The findings suggest that this patient did not benefit from baclofen. Attendant opinion was that she had 'clinically' improved - i.e., they could do more with her than before and discovered more and more activities she liked. Control of her epilepsy had not deteriorated.

- Patient 7, a 32-year old severely retarded woman with little verbal communication had dyskinetic quadriplegia of unknown etiology. Her motor handicap included choreo-athetosis and dystonia; the latter was greatly influenced by her emotional status. She was practically chair bound and only with a lot of support could she take a few steps. In her wheelchair she made attempts to move about independently. For the past 7 years she had been treated with baclofen, 45 mg daily (0.21 mg.l $^{-1}$). She also received diazepam 6 mg daily, and an oral contraceptive. The aim was to maintain her present arm and hand function as well as ease of management. As shown in figure 3, during the entire period of baclofen reduction, minor changes in the mean ADL score were found, which extended to the drug-free period. On the video scale, the period of no drug could be distinguished from that of initial medication by an improvement in the score; for the 20-mg dose, an intermediate value was found. Video rank order varied between the observers. The findings of both evaluation methods agreed that this patient did not perform better while on baclofen. Variability in the ADL data was considerable but not surprising with dystonia as a major handicap. On the 20 mg dose, the plasma concentration was low (0.03 mg.l $^{-1}$) and the clearance high (467 ml.min $^{-1}$). Improvement of mood coincided with baclofen reduction, but not as remarkably as for patient 3.

- Patient 8, a 44-year old moderately retarded woman with no verbal communication had dyskinetic quadriplegia of unknown etiology. Her motor handicap included rigidity, dystonia, and athetosis; spastic involvement was apparent in the legs. She had developed flexion contrac-

tures and was not ambulant. She could move about independently in an electrically-driven wheelchair. For the past 5 years she had been treated with baclofen, 30 mg daily (0.15 mg.l $^{-1}$). Alternatively, orphenadrine and ipratropium were given against excessive salivation. She further received bisacodyl and lactulose. The aim was to maintain her present arm function as well as ease of management. Prior to medication reduction, the effect of increased dosage was investigated. As shown in figure 3, during the entire period of dosage alterations, only small changes in the mean ADL score were found. On the video scale, the period of increased dosage could be distinguished from the period of initial medication and that of no drug by a deterioration in the score, which agreed with video rank order. On the 60-mg dose, the plasma concentration was 0.31 mg.l $^{-1}$, the clearance 134 ml.min $^{-1}$ The findings of both evaluation methods agreed that this patient did not perform better while on baclofen. Attendant opinion was that operating her wheelchair had slightly improved on the high dose but at the expense of hanging extremely limply in her chair. As for patient 7, variability in the ADL data was considerable but not unexpected. Her bad temper, on and off a problem throughout the entire period, made her a difficult patient for testing and may have added to the variability.

- Patient 9, a 21-year-old mildly retarded woman with good language function had spastic quadriplegia resulting from prenatal pregnancy toxicosis. With support she was ambulant and with an electrically-driven wheelchair she could move about independently. For the past 6 years she had been treated with dantrolene, 75 mg daily; the dantrolene plasma concentration was 0.44 mg.l-1, the concentration of 5-hydroxy-dantrolene 0.14 mg.l $^{-1}$. She also received baclofen, 30 mg daily, which was kept unchanged; the baclofen plasma concentration was 0.20 mg.l $^{-1}$. Lactulose was the only other drug used. The major aim was to maintain her present walking ability. As shown in figure 4, performance on the ADL scale was slightly better on low dosage or no drug, which had similar means. On the video scale, the dantrolene-free period could be distinguished by a deterioration in the score from the initial medication, although the difference was small. For 2 of the 3 observers, the rank order followed the video score. On the 23-mg dose, the dantrolene plasma concentration was 0.10 mg.l$^{-1}$ (CL/f 126 ml.min$^{-1}$); the concentration

of 5-hydroxydantrolene was below 0.02 mg.l $^{-1}$, which is the detection limit of the assay. Whether dantrolene was of benefit for her locomotive behaviour remains unclear. After drug withdrawal, dyskinetic involvement became more apparent, notably dystonia in neck and left arm. This finding agreed with attendant opinion that the way she operated her wheelchair had become more variable. The baclofen plasma concentration had not changed.

- Patient 10, a 12-year-old mildly retarded girl with good language function and poor vision had spastic quadriplegia resulting from birth asphyxia. She was practically chair-bound and only with a lot of support could she take a few steps. For the past 6 years she had been treated with dantrolene, most recently with 75 mg daily; the dantrolene plasma concentration was 0.33 mg.l $^{-1}$, the metabolite concentration 0 08 mg.l $^{-1}$. She also received baclofen 45 mg daily, which was kept unchanged; the plasma concentration was 0.31 mg.l$^{-1}$. Her epilepsy was held under control with carbamazepine 400 mg daily (8.5 mg.l $^{-1}$). The major aim was to maintain ease of management. As shown in figure 4, on the ADL scale performance was best on low dosage or no drug, which had almost similar means. On the video scale, the period of initial medication could not be distinguished from the dantrolene-free period. Video rank order varied between the observers. On the 15-mg dose, the dantrolene plasma concentration was 0.04 mg.l$^{-1}$ (CL/f 205 ml.min $^{-1}$); the concentration of the metabolite escaped detection. The findings of both evaluation methods agreed that this patient did not perform better while on dantrolene. After drug withdrawal, dyskinetic involvement became more distinct: dystonia and abnormal movement patterns of a plastic rigid type in neck, trunk, arms, and hands. The baclofen plasma concentration had not changed.

- Patient 11, a 13-year-old profoundly retarded girl with no verbal communication had spastic quadriplegia of unknown etiology. Dystonia was apparent in the neck. Particularly disabling was the retention of primitive responses. She was chair-bound with hardly any voluntary control and had minor contractures. She had been treated with dantrolene for practically her whole life, most recently with 75 mg daily; the

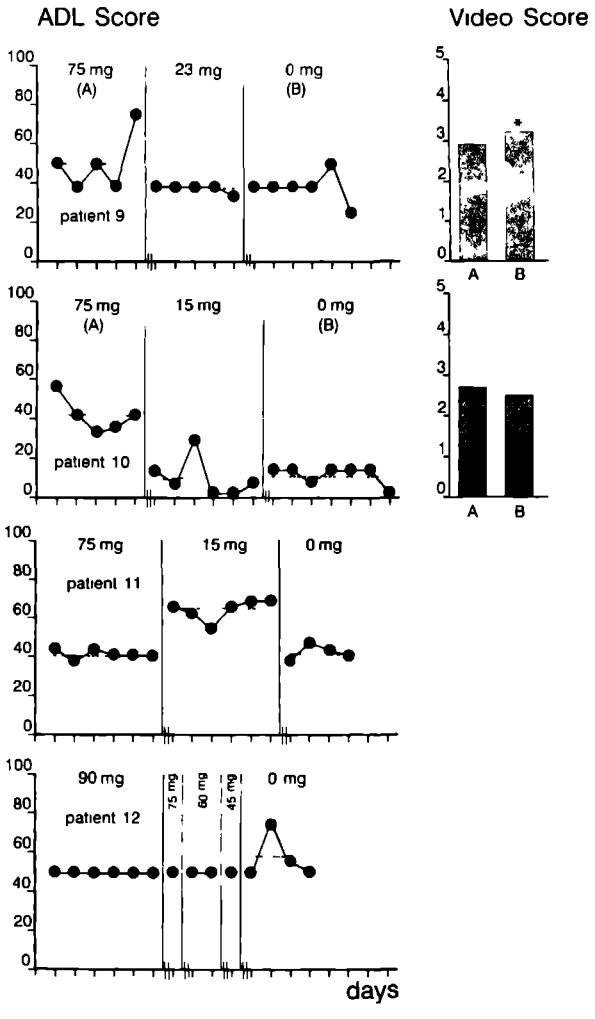


FIGURE 4

ADL (line graphs) and video (histograms) scores for 4 spastic patients (9-12) during dantrolene reduction. In patient 9 the walking ability, in the other patients ease of management or passive movement was tested. Dashed lines as in figure 2.

*qualified as deteriorated

dantrolene plasma concentration was 0.42 mg.l $^{-1}$, the concentration of the metabolite 0.13 mg.l $^{-1}$. She also received baclofen, 30 mg daily, which was kept unchanged; the plasma concentration was 0.34 mg.l $^{-1}$. The major aim was to maintain ease of management. As shown in figure 4, the mean ADL score for the dantrolene-free period was similar to the initial mean value. On the 15 mg dose, a deterioration in the score was found. For this dose, the dantrolene plasma concentration was 0.21 mg. l $^{-1}$ (CL/f 39 ml.min $^{-1}$); the metabolite concentration escaped detection. The findings suggest that the daily care of this patient was not made easier while she was on dantrolene. A period of frequent crying coincided with the 15-mg phase that might have affected the rating. In the opinion of the attendants, she had become a little more stiff since being off the drug. The baclofen plasma concentration had not changed.

- Patient 12, a 16-year-old profoundly retarded blind girl with no verbal communication had spastic quadriplegia of unknown etiology. Slight dyskinetic involvement was apparent in the face (mouth). She was bedridden with almost no voluntary movement or control of posture and had fixed deformities. For the past 5 years she had been treated with dantrolene, most recently with 90 mg daily; the dantrolene plasma concentration was 0.43 mg.l $^{-1}$, the concentration of the metabolite 0.14 mg.l $^{-1}$. She also received baclofen 30 mg daily, which was kept unchanged; the plasma concentration was 0.15 mg.l $^{-1}$. Her epilepsy was held under control with carbamazepine 400 mg daily (8 mg.l $^{-1}$). She further received acetylcysteine, bisacodyl, and lactulose. The aim was to maintain the present range of passive motion in her arms and legs. As shown in figure 4, across the different phases of reduced dosage no change in the score as recorded by the physiotherapist was found. On the 60-mg dose, the dantrolene plasma concentration was 0.29 mg.l $^{-1}$ (CL/f 113 ml.min $^{-1}$), the concentration of 5-hydroxydantrolene 0.11 mg. l $^{-1}$. For the drug-free period, a slight deterioration in the score was found. The findings suggest almost no influence of dantrolene on the ease of handling this patient. The baclofen plasma concentration had not changed.

In this pilot study with mentally retarded cerebral palsied patients, we measured muscle tone as the variable related to the pharmacological activity of the drugs investigated, baclofen and dantrolene. The increase and decrease in tone seen following withdrawal of either drug suggest, however, no influence on this variable. Possible explanations are that the normal variation in tone in these patients has masked an effect (16) or that the plasma concentrations were too low to exert muscle relaxation. Pharmacological activity after prolonged administration of baclofen in spinal spasticity has previously been described with highly varying concentrations that encompassed the values reported here (17). In patients with spasticity of different origin, dantrolene plasma concentrations of over 0.3 mg.l $^{-1}$ have been associated with muscle relaxation (18,19).

Posture and movement abnormalities were measured on a simple scale that could be used for all patients irrespective of the degree of their mental or motor handicap. The results of this test suggest that baclofen and dantrolene did have some effect on the motor manifestations of spastic cerebral palsy. The initial 'active' plasma concentrations varied between 0.12 and 0.24 mg.l $^{-1}$ for baclofen, and between 0.33 and 0.44 mg l $^{-1}$ for dantrolene. For a meaningful interpretation of these numbers one must realise that the drug taken may either not be a single active compound or remain just one active compound. The active hydroxy metabolite of dantrolene, which was simultaneously measured in this study, was present in concentrations that corresponded to an average parent drug/metabolite ratio of 3, which agreed with the Meyler study (19). In the case of baclofen, the clinically available dosage form contains the racemate - i.e., 50% of the active (-)-(R)-enantiomer and 50% of the inactive (+)-(S) enantiomer (20), a ratio which may be subjective to change in the body. The assay used in the present study did not discriminate between the two. Following a single oral dose in a healthy subject, it has been shown that differences in the plasma profiles of these isomers were small (21).

The present study also attempted to document changes in everyday motor performance of individual patients. Repeated assessments of simple daily tasks by a familiar attendant seemed to be the most practical way

to measure the effect of medication reduction, supported by incidental video recordings for objectiveness. However, changes across the different treatment phases were minor, absent, or inconsistent, and did not relate to the plasma concentrations in the ranges measured. Whether the patients themselves experienced differences in performance, could not be tracked in this severely handicapped group.

Except for a number of samples measured during medication reduction, the plasma concentrations did show a good correlation with the dosages administered. For baclofen, the mean total body clearance agreed with the mean value found for hospitalised spastic patients where multiple samples during a dosage interval were available for clearance calculations (22). This observation also holds for the patients where baclofen was present as co-medication. Clearance values for dantrolene, have not been previously published. A poor concentration-dose relationship was found by Inotsume et al. (23), who measured plasma concentrations following widely varying daily dosages. From the data reported for patients treated similarly as the cases presented here (19), clearances could be derived which agreed with the present values.

Abrupt withdrawal of baclofen after prolonged treatment has been associated with hallucinations and seizures (11). Even though baclofen was gradually withdrawn in this study, increased incidence of seizures occurred in two of the six patients with associated epilepsy. Concomitant reduction in anticonvulsant dosage and level may have played a role in patient 1. Baclofen may also have acted as an anticonvulsant in the patients 1 and 2, but this aspect of baclofen therapy is still controversial (24). Withdrawal symptoms have not been reported previously for dantrolene. In two of the four patients who had their dantrolene withdrawn, we observed an increase in dyskinetic involvement. Re-appearance of pre-existent motor impairment, however, should also be considered as a possibility, since dantrolene has been reported to be useful in patients with dyskinetic cerebral palsy (5,6).

In many aspects the present study was a pilot study, in which the feasibility of studying concentration-effect relationships of the antispastic drugs baclofen and dantrolene in mentally retarded cerebral palsied patients was investigated. Future research efforts should include both plasma concentrations and pharmacological and clinical variables repeatedly measured over time under 'single-case research' condi-

tions (15,25), assisted by enantioselective baclofen measurements, and extended to cover other dosage regimens.

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## Summary and conclusions

A clinical question stimulated the pharmacokinetic investigation of drugs which are effective in the management of patients with spasticity. Patients suffering from this disorder of motor control may have to take these drugs daily for extended periods or even for their whole life. Physicians in charge of such patients wondered whether plasma concentration measurements could guide their prescribing habits for spasticity, similar to the practice now common for epilepsy. The neurological disorders resemble each other inasmuch that therapeutic response to a given treatment is often hard to evaluate.

Of the muscle relaxants which have been developed over the years, baclofen and dantrolene are established antispastic drugs. Although both have been in use since 1967, pharmacokinetic investigations of either drug have been limited, which is partly explained by the lack of suitable assays.

The purpose of the present study was to develop chemical analyses for the quantitative determination of baclofen and dantrolene, and to apply these to study the pharmacokinetics of the drugs. The work is described in thirteen articles.

## LITERATURE REVIEW

In **chapter 1** the relevant characteristics of baclofen and dantrolene are reviewed, including their place in the treatment of spasticity. Both drugs differ completely in their chemical structure and mode of action. The $GABA_B$ agonist baclofen is a centrally acting muscle relaxant. The drug is marketed as the racemate, but the (-)-(R)-enantiomer has proved to be the active isomer. Dantrolene, a structural analogue of nitrofurantoin, is a directly acting muscle relaxant, which is extensively metabolised in the body.

In addition, this chapter deals with several characteristics of the spasticity syndrome, because insight into classification systems, methods of measurement etc. is of importance when one wishes to relate plasma concentration to therapeutic response. The meaning of the term spasticity is also briefly discussed. The term 'cerebral palsy' is in-

cluded in the discussion, because it is also referred to as spasticity, and is the particular field of clinical investigation described in chapter 13.

## ANALYTICAL PROCEDURES

In **chapter 2** a high-performance liquid chromatographic (HPLC) method for the quantitative determination of baclofen in plasma and urine is described. The problem of detecting nanogrammes of a substance with an amino acid structure among the abundantly present endogenous amino acids became known as finding an invisible needle in an invisible hay stack. Derivatization with o-phthaldialdehyde reduced the problem to that of the needle in the haystack: the highly fluorescent isoindole corresponding to the amino acid (including the p chlorophenyl derivative of $\gamma$ aminobutyric acid, thus baclofen) is synthetised in the presence of alkylmercapto compounds such as thioglycolic acid. Interfering amino acids were efficiently removed by cation exchange extraction prior to chromatographic separation. An on column concentration and cleaning procedure permitted the injection of the large volumes needed to obtain the required sensitivity. Reversed-phase HPLC with fluorimetric detection (excitation at 340 nm, emission at 460 nm) completed the procedure. With this method, which was demonstrated to be suitable for pharmacokinetic purposes, baclofen could be measured in 0.5 ml of plasma or urine with a detection limit of 1-2 ng, a precision better than 6%, and a recovery greater than 90%.

Although this method was suitable, a simpler method for high concentrations of baclofen in urine was developed which is described in **chapter 3.** Upon direct injection in a reversed-phase HPLC system with ultraviolet detection (220 nm), baclofen can be measured in samples with concentrations over 4 $mg.l^{-1}$, levels we never encountered in plasma. The proposed $\gamma$ hydroxy metabolite of baclofen, which escapes detection in the o-phthaldialdehyde fluorimetric procedure because it lacks the $NH_2$-group required for derivatization, can also be measured with this method if present in the same concentrations.

In **chapter 4** the analytical problem that racemic 'fixed-ratio' combination drugs like baclofen present is tackled. Since the chiral drug baclofen is marketed as the racemate, it follows that, to continue the metaphor, not one but two needles - one the mirror image of the other - were concealed in the haystack. Chiral separation was eventually obtained by substituting N-acetyl-L-cysteine, an optically active alkylmercapto compound, for the achiral thioglycolic acid. The o-phthaldialdehyde derivatization reaction having thus been modified, the formed diastereoisomers could be determined quantitatively, for the present in aqueous media.

In **chapter 5** a method for the quantitative determination of dantrolene and two of its metabolites in plasma and urine is described. The poor solubility of the compounds in water (the metabolites included!) and in many other solvents, and the degradation by light are complicating factors in the analysis. Plasma had to be extracted prior to the chromatographic separation. A mixture of chloroform-n-butanol (95:5) yielded good recoveries for all three compounds. Urine could be directly injected. With reversed-phase HPLC followed by ultraviolet detection (375 nm), dantrolene, 5-hydroxydantrolene, and nitro-reduced acetylated dantrolene (also known as acetylaminodantrolene) could be measured simultaneously in a single isocratic run of about 10 min with a detection limit of 1 ng, thus providing a method which is suitable for pharmacokinetic studies.

In **chapter 6** a modification of the method is presented which was developed to determine the metabolite which results from azomethine cleavage, notably 5-(p-nitrophenyl)-2-furoic acid. In the extraction procedure of chapter 5, this compound appeared to have been discarded with the aqueous layer. Acidification prior to extraction, chromatographic separation with a slightly different mobile phase, and detection at a wavelength of 354 nm permitted determination of this cleavage product in plasma (and urine). Preliminary pharmacokinetic experiments demonstrated that this metabolite is probably not important in man, but is in the dog.

**Chapter 7.** The pharmacokinetics of racemic baclofen and its separate enantiomers were studied in dogs. For this part of the study laboratory animals were chosen because no data were available on the toxicity of the isomers when separately administered. Because of the resemblance between the human and the canine kidney, the dog was chosen. Renal parameters in pharmacokinetic experiments add information and are indispensable for a drug like baclofen, which has caused signs of intoxication in patients with renal function impairment. After a single intravenous infusion, differences were found in the urinary recovery of the compounds. For the active (-)-(R)-enantiomer, the mass balance was almost complete as 85% was recovered. To eliminate (+) (S)-baclofen, more excretion pathways seem to be involved. The findings suggest that the active enantiomer is also to be preferred with respect to the pharmacokinetic factors. Irrespective of isomeric composition, the apparent renal clearance of baclofen was found to be related to the creatinine clearance.

**Chapter 8** deals with the pharmacokinetics of baclofen in healthy subjects after oral administration of the clinically available dosage form containing the racemate. Following a single 40 mg dose, baclofen was for the greater part excreted unchanged by the kidney within two days, on the average, 70% of the dose. The mean half-life, calculated from extended least squares modelling (ELSMOS) both of plasma and urine data was 7 h, which was longer than reported in most studies based solely on plasma data. The apparent renal clearance of baclofen equalled the creatinine clearance. Analogous to the findings in the dog, the assumption is that this relationship will not be stereoselective. Although active tubular secretion may contribute to baclofen's renal clearance as shown by the effect of co-administered probenecid, glomerular filtration appears to be the dominant transport mechanism.

In **chapter 9** the pharmacokinetics of baclofen in spastic patients treated with individualised oral doses of tablets, containing the racemate, are presented. Relative to the healthy subjects, sampling opportunities were rather limited. The ailment itself made quantitative

urine collection a difficult task. The sampling period was also much shorter, i.e. a dosage interval (q6h or q8h) for plasma, because drug withdrawal was not attempted in view of the risk of adverse reactions The fluctuation found in the plasma concentration was considerable, 200 to 400%, though consistent with the half-life calculated in chapter 8 The percentage of the dose excreted unchanged by the kidney was also similar to the values found for the healthy volunteers after a single dose. In the light of the findings in the previous chapters, the view is offered that the observed variation in the apparent renal clearance of baclofen is likely to be related to the variation in the creatinine clearance. The contribution of the renal clearance to the total body clearance can explain the described toxicity when renal function impairment is present.

PHARMACOKINETICS OF DANTROLENE

**Chapter 10** describes the distribution of $^{14}C$-labelled dantrolene in the marmoset monkey as studied by whole-body autoradiography. This laboratory animal was chosen because it is a small primate. At steady state following linear infusion, high accumulations of radioactivity were found in the kidneys, liver, gall bladder, urinary bladder, and intestines, i.e. in organs concerned with the elimination of dantrolene. Much lower concentrations were observed in the skeletal muscles, the site of dantrolene's action. Only traces of radioactivity were present in the brain, suggesting that the intact molecule cannot pass the blood-brain barrier. The position of the $^{14}C$-label prevented possible products of azomethine cleavage, notably hydantoin derivatives, to be visualised.

**Chapter 11** deals with the pharmacokinetics of dantrolene and its active metabolite, 5-hydroxydantrolene, in the dog. To circumvent bioavailability problems, the substances were intravenously administered. Because of this approach and the intended sampling of bile, laboratory animals were preferred to human volunteers. Despite the intravenous route, the mass balance was far from complete. Only the hydroxy compound was recovered in quantity in the urine (2%) and in the bile

(25%). Bile to plasma ratios of this metabolite were high with biliary concentrations far exceeding the maximum solubility in water. The half-life of 5-hydroxydantrolene was shorter than that of dantrolene as demonstrated by administration of the metabolite. These findings indicate that, in the dog, hydroxylation is important in dantrolene excretion from the body.

An example of the pharmacokinetics of dantrolene in man is given in **chapter 12**, where a case report on oral prophylaxis for malignant hyperthermia is presented. In addition to dantrolene's use in spasticity, it is the drug of choice for this syndrome. Parent drug and hydroxy metabolite both declined with a half-life of 15 h, which agrees with the findings in the dog.

## PHARMACOKINETICS AND SPASTICITY

**Chapter 13** describes the results of a pilot study on the effects of baclofen and dantrolene in a group of institutionalised patients with cerebral palsy. For assessment of the motor handicap tests were developed, which had to be very simple because of the associated mental retardation. For patients being treated with antispastic drugs and suspected of having no (optimal) clinical benefit, a reduction in medication was evaluated while monitoring plasma concentration and effect. From the cases presented, the feasibility of a study on concentration-effect relationships in this particular patient population is discussed.

## CONCLUDING REMARKS

The investigations carried out and reported in this thesis were done in order to obtain insight into the pharmacokinetics of antispastic drugs. The assay developed for baclofen was sufficiently sensitive for measuring concentrations in plasma and urine following therapeutic doses. Because of the high urinary concentrations, a simplified procedure could be used for most urine samples. The first results of the

enantioselective analyses should lead to methods which are applicable to biological material. The γ-hydroxy metabolite of baclofen is probably of no importance in man and dog. Our findings were in favour of a correlation between the creatinine clearance and the renal clearance of unchanged baclofen, irrespective of isomeric composition. Studies with enantioselective assays are required to examine the pharmacokinetics of baclofen in more detail.

The assay developed for dantrolene was sufficiently sensitive and specific for the quantitative determination of dantrolene and two of its metabolites. The method was applicable to plasma, urine, and bile. Contrary to the findings in dogs, the drug and its metabolites could not be detected in human bile. A product of azomethine cleavage did not contribute to the incomplete mass balance of dantrolene in man. Our findings are in favour of the concept that hydroxylation diminishes the half-life of this drug. Future studies will have to focus upon metabolite kinetics.

The clinical findings indicate how information correlating plasma concentration to effect in patients with cerebral palsy might be obtained, particularly in coexistent mental retardation. Other antispastic drugs such as tizanidine, which has more recently become available, may be similarly tested. Because of the racemic character of clinically applied baclofen and the incomplete mass balance of dantrolene, it may not come as a surprise that no clear recommendations can yet be given on concentration-effect relationships. A spin-off of the pilot study in the institution concerned is that the prescribing of antispastic drugs has remarkably declined.

# FARMACOKINETIEK VAN ANTISPASTICA
## een studie over baclofen en dantroleen

Een klinische vraagstelling is de aanleiding geweest tot het hier beschreven onderzoek naar de farmacokinetiek van geneesmiddelen die worden toegepast bij de behandeling van patienten met spasticiteit. Deze patienten, die zich presenteren met stoornissen in de motoriek, moeten soms jarenlang (levenslang?) dagelijks geneesmiddelen innemen. Artsen belast met de zorg voor dergelijke patienten kwamen met de vraag of het meten van geneesmiddelconcentraties in plasma hun voorschrijfpatroon bij dit ziektebeeld zou kunnen beinvloeden. Met deze aanpak hadden ze goede ervaringen opgedaan bij het behandelen van patienten met epilepsie, een neurologische aandoening die in zoverre vergelijkbaar is met spasticiteit, dat het effect van een bepaalde therapie eveneens vaak moeilijk is te beoordelen.

Van de verschillende spierverslappers die in de loop der jaren zijn ontwikkeld, hebben baclofen en dantroleen zich een plaats verworven bij de behandeling van spasticiteit. In navolging van de Angelsaksische literatuur zijn spierverslappers met dit toepassingsgebied hier antispastica genoemd. Hoewel baclofen en dantroleen al vanaf 1967 worden gebruikt, zijn farmacokinetische gegevens nog maar spaarzaam beschikbaar, hetgeen deels kan worden verklaard door het ontbreken van geschikte bepalingsmethoden. Dit leidde tot het formuleren van de volgende doelstellingen: het ontwikkelen van chemische analyses voor de kwantitatieve bepaling van baclofen en dantroleen, en het bestuderen van de farmacokinetiek van beide antispastica middels deze analyses. Het onderhavige werk is een bundeling van dertien artikelen over dit onderwerp.

## LITERATUUROVERZICHT

In **hoofdstuk** 1 wordt een literatuuroverzicht gegeven van enige kenmerkende eigenschappen van baclofen en dantroleen, met inbegrip van hun plaats bij de behandeling van spasticiteit. Beide middelen zijn volkomen van elkaar verschillend qua chemische structuur en werkingswijze.

De $GABA_B$-agonist baclofen is een centraal-werkend spierrelaxans, dat in de handel is als racemisch mengsel, maar waarvan slechts een enantiomeer, de (-)-(R)-isomeer, verantwoordelijk is voor de werking. Dantroleen, een verbinding die structuurovereenkomst vertoont met nitrofurantoine, is een direct-werkende spierverslapper die sterk onderhevig is aan metabole afbraak.

Dit hoofdstuk behandelt verder een aantal kenmerken van het syndroom spasticiteit, omdat enig inzicht in classificatiesystemen, meetmethoden e.d. van belang is als men verbanden wil gaan leggen tussen plasmaconcentraties en therapeutisch effect. Er wordt tevens kort ingegaan op wat allemaal onder de tamelijk verwarrende term spasticiteit wordt verstaan. Zo wordt het ziektebeeld infantiele encefalopathie, dat in de Angelsaksische literatuur bekend staat als 'cerebral palsy', ook met de term spasticiteit aangeduid. Het in hoofdstuk 13 beschreven klinische onderzoek heeft betrekking op patienten met deze specifieke aandoening.

## ANALYTISCHE BEPALINGSMETHODEN

In **hoofdstuk 2** wordt een methode voor de kwantitatieve bepaling van baclofen in plasma en urine met behulp van hoge-drukvloeistofchromatografie (HPLC) beschreven. Het probleem om nanogrammen van een stof met een aminozuurachtige structuur aan te tonen temidden van de overvloedig aanwezige endogene aminozuren, is te vergelijken met het zoeken naar een onzichtbare speld in een onzichtbare hooiberg. Derivatisering met o-ftaaldialdehyde bracht het probleem terug tot dat van de bekende speld in de hooiberg: in aanwezigheid van alkylmercaptoverbindingen (in dit geval thioglycolzuur) worden aminozuren, met inbegrip van het p-chloorfenylderivaat van $\gamma$-aminoboterzuur, te weten baclofen, omgezet in sterk fluorescerende isoindolen. Extractie via een kationuitwisselingsprocedure voorafgaande aan chromatografische scheiding bleek interfererende aminozuren effectief te kunnen verwijderen. Met behulp van een concentratiekolom werd een verdere concentratie en opschoning van de monsters verkregen. De relatief grote volumina die zo konden worden opgebracht, droegen bij aan de vereiste gevoeligheid. 'Reversed-phase' HPLC met fluorimetrische detectie (excitatie bij 340 nm, emissie bij 460 nm) completeerde de analysegang. Met deze methode was het mogelijk

om uitgaande van 0,5 ml monster baclofen in plasma of urine te bepalen met een detectielimiet van 1-2 ng, een nauwkeurigheid van tenminste 6% en een opbrengst van ruim 90%, hetgeen geschikt bleek te zijn voor farmacokinetische doeleinden.

Alhoewel geschikt, werd daarnaast een minder bewerkelijke methode voor het bepalen van hoge concentraties baclofen in urine ontwikkeld (**hoofdstuk 3**). Via directe injectie in een reversed-phase HPLC-systeem met ultraviolet-detectie (220 nm) was het mogelijk monsters met baclofenconcentraties van meer dan 4 mg.l $^{-1}$ te meten. Dergelijk hoge waarden werden nooit aangetroffen in plasma. Een mogelijke metaboliet van baclofen, 3-(p-chloorfenyl)-4-hydroxyboterzuur, die men mist bij de derivatiseringsreactie met o-ftaaldialdehyde wegens afwezigheid van de hiervoor benodigde $NH_2$-groep, kan ook met deze methode worden gemeten, mits aanwezig in even hoge concentraties.

In **hoofdstuk 4** wordt de analytische problematiek die men met chirale geneesmiddelen zoals baclofen in huis haalt, aangepakt. Aangezien de handelsvorm van baclofen, zoals eerder opgemerkt, het racemische mengsel bevat, waren er kennelijk niet een maar twee spelden verborgen in de hooiberg, zijnde elkaar's spiegelbeeld. Chirale scheiding werd uit eindelijk verkregen door het achirale thioglycolzuur te vervangen door de optisch actieve alkylmercaptoverbinding N-acetyl-L-cysteine. Met deze modificatie van de o-ftaaldialdehyde-derivatiseringsreactie konden de gevormde diastereoisomeren kwantitatief, vooralsnog in waterig milieu, worden bepaald.

In **hoofdstuk 5** wordt een methode voor de kwantitatieve bepaling van dantroleen en twee van zijn metabolieten in plasma en urine beschreven. Complicerende factoren bij de analyse vormden de uitermate slechte oplosbaarheid van deze verbindingen in water (ook de metabolieten!) en vele andere oplosmiddelen, alsmede de ontleding onder invloed van licht. Plasma werd geextraheerd met een mengsel van chloroform-n-butanol (95:5), waarmee een goede opbrengst voor alle drie de verbindingen werd verkregen; urine behoefde geen verdere voorbewerking. Met behulp van reversed-phase HPLC gevolgd door ultraviolet-detectie (375 nm) was het mogelijk dantroleen, 5-hydroxydantroleen en acetylaminodantroleen

in een enkele isocratische analysegang van ongeveer 10 min te analyseren met een detectielimiet van 1 ng, hetgeen de methode geschikt maakte voor farmacokinetische studies.

Een modificatie van deze methode is opgenomen in **hoofdstuk 6**. Met de bovenbeschreven extractieprocedure bleek namelijk de metaboliet die na verbreking van de azomethinebinding in het dantroleenmolecuul ontstaat, het 5-(p-nitrofenyl)-2-furaanzuur, met de waterige laag te zijn verdwenen. Extractie na aanzuring en enkele kleine aanpassingen van mobiele fase en golflengte (354 nm) maakten het mogelijk ook deze verbinding in plasma (en urine) te bepalen. Uit de eerste resultaten van farmacokinetisch onderzoek blijkt dat deze metaboliet vermoedelijk niet van belang is bij de mens, maar wel bij de hond.

FARMACOKINETIEK VAN BACLOFEN

**Hoofdstuk 7.** De farmacokinetiek van racemisch baclofen en van elk enantiomeer afzonderlijk werd bestudeerd bij de hond. Tot een onderzoek met proefdieren werd besloten, omdat toxiciteitsgegevens over de separaat toegediende isomeren ontbraken. De keuze voor de hond werd mede ingegeven door de overeenkomst in nier tussen mens en hond. Het betrekken van renale parameters bij farmacokinetische experimenten verschaft veel extra informatie en is onontbeerlijk voor een geneesmiddel als baclofen, waarvan bekend is dat verschijnselen van intoxicatie kunnen optreden bij verminderde nierfunctie. Na een eenmalige intraveneuze infusie werden onder meer verschillen geconstateerd in de hoeveelheid renaal uitgescheiden stof. Voor de actieve (-)-(R)-enantiomeer was de massabalans bijna compleet met een recovery van 85%. Voor het verwijderen van (+)-(S)-baclofen schijnen verschillende eliminatieroutes verantwoordelijk te zijn. De bevindingen suggereren dat werkzaamheid en een gunstig farmacokinetisch profiel in het geval van baclofen samengaan. De samenhang die werd gevonden tussen de schijnbare renale klaring van baclofen en de creatinineklaring, bleek onafhankelijk te zijn van de isomere samenstelling van de toegediende verbindingen.

**Hoofdstuk 8** handelt over de farmacokinetiek van baclofen bij gezonde proefpersonen na orale inname van de in de handel zijnde doseervorm, dwz. tabletten met het racemaat. Na een eenmalige dosis van 40 mg werd binnen twee dagen gemiddeld 70% van de toegediende hoeveelheid onveranderd teruggevonden in de urine. De halfwaardetijd die werd berekend uit het gecombineerd fitten van plasma- en urinegegevens met behulp van 'extended least squares modelling' (ELSMOS), bedroeg gemiddeld 7 uur. Dit is langer dan gerapporteerd in de meeste andere onderzoeken die zich alleen op plasmagegevens baseerden. De schijnbare renale klaring van baclofen was gelijk aan de creatinineklaring. Gezien de bevindingen bij de hond kan men veronderstellen dat dit verband niet stereoselectief zal zijn. Hoewel actieve tubulaire uitscheiding een bijdrage kan leveren aan de renale klaring, zoals werd aangetoond met het toedienen van probenecide, is het aannemelijk dat glomerulaire filtratie een overheersende rol speelt bij het transport van baclofen via de nier.

De farmacokinetiek van baclofen bij spastische patienten die met individuele onderhoudsdoses in de vorm van tabletten met het racemaat werden behandeld, is het onderwerp van **hoofdstuk 9**. Vergeleken met de bovenbeschreven gezonde vrijwilligers was monstername maar zeer beperkt mogelijk. De aandoening zelf was ervoor verantwoordelijk dat het kwantitatief verzamelen van urine moeizaam verliep. De totale periode van monstername was ook beduidend korter, nl. een doseringsinterval (6 of 8 uur) in het geval van plasma, omdat in verband met de kans op onttrekkingsverschijnselen stoppen met de therapie niet verantwoord werd geacht. De aanzienlijke fluctuatie die in de plasmaconcentratie werd gevonden, 200 tot 400%, paste goed bij de halfwaardetijd zoals berekend in hoofdstuk 8. Het percentage van de dosis dat onveranderd in de urine werd aangetroffen, was eveneens niet afwijkend van de waarden na eenmalige toediening aan proefpersonen. Op grond van de bevindingen in de beide vorige hoofdstukken wordt de variatie in de schijnbare renale klaring van baclofen met de variatie in de creatinineklaring in verband gebracht. De bijdrage die de renale klaring levert aan de plasmaklaring, kan een verklaring vormen voor de beschreven toxiciteit bij patienten met verminderde nierfunctie.

**Hoofdstuk 10** beschrijft de verdeling van $^{14}$C-gemerkt dantroleen bij de marmosetaap zoals bestudeerd met behulp van macroautoradiografie. Dit proefdier werd gekozen omdat het een kleine primaat is. Na lineaire infusie tot 'steady-state' werd onder meer een sterke ophoping van radioactiviteit gevonden in de nier, lever, galblaas, urineblaas en darmen, dwz. in organen die in verband kunnen worden gebracht met de eliminatie van dantroleen. Op de plaats van werking, de skeletspieren, werden veel lagere concentraties aangetroffen. Slechts sporen radioactiviteit waren aanwezig in de hersenen, hetgeen suggereert dat het intacte molecuul de bloed-hersenbarrière niet kan passeren. Door de positie van het $^{14}$C-label konden eventuele ontledingsprodukten ten gevolge van het verbreken van de azomethinebinding, met name afgeleiden van hydantoine, niet worden waargenomen.

**Hoofdstuk 11.** De farmacokinetiek van dantroleen en 5-hydroxydantroleen, een actieve metaboliet, werd bestudeerd bij de hond. Om mogelijke problemen van biologische beschikbaarheid te vermijden, werden de verbindingen intraveneus toegediend. Vanwege deze benadering werd de voorvoorkeur gegeven aan proefdieren, hetgeen tevens een goede mogelijkheid bood om uitscheiding via de gal te onderzoeken. Ondanks de intraveneuze toediening werd een verre van volledige massabalans gevonden. Alleen de hydroxyverbinding werd in redelijke hoeveelheden teruggevonden in de urine (2%) en in de gal (25%). Vergeleken met plasma waren de concentraties van 5-hydroxydantroleen in de gal zeer hoog, waarbij de maximale wateroplosbaarheid ver werd overschreden. Door het toedienen van de metaboliet als moederverbinding kon worden aangetoond, dat met hydroxylering de halfwaardetijd afneemt. Uit deze bevindingen mag worden afgeleid, dat hydroxylering een belangrijke rol speelt bij het verwijderen van dantroleen uit het lichaam van de hond.

**Hoofdstuk 12.** De farmacokinetiek van dantroleen bij de mens wordt beschreven aan de hand van een geval van profylactisch gebruik bij maligne hyperthermie. Behalve spasticiteit is het syndroom maligne hyperthermie het indicatiegebied voor dantroleen geworden. Na orale voorbehandeling waren de halfwaardetijden van moederverbinding en hydroxyme-

taboliet beide 15 uur, hetgeen in overeenstemming is te brengen met de bevindingen bij de hond.

FARMACOKINETIEK EN SPASTICITEIT

**Hoofdstuk 13** beschrijft een verkennende studie naar de effecten van baclofen en dantroleen bij een groep patienten met cerebral palsy, die waren gehuisvest in een zwakzinnigeninstituut. Voor het registreren van het motorisch functioneren werden evaluatiemethoden ontwikkeld, die zeer eenvoudig moesten zijn vanwege de eveneens aanwezige geestelijke handicap. Bij patienten die met antispastica werden behandeld en bij wie het vermoeden bestond dat de huidige therapie niet (optimaal) effectief was, werd de medicatie verminderd onder controle van plasmaconcentratie en effect. Aan de hand van de gepresenteerde casussen wordt de haalbaarheid van een studie naar concentratie-effect relaties bij dergelijke patienten aangegeven.

SLOTOPMERKINGEN

Het in dit proefschrift beschreven onderzoek werd uitgevoerd om inzicht te verkrijgen in de farmacokinetiek van antispastica. De voor baclofen ontwikkelde analysemethode bleek voldoende gevoelig te zijn voor het kwantitatief meten van plasma- en urineconcentraties na therapeutische doses. Een sterk vereenvoudigde procedure kon worden gebruikt voor de hoge concentraties die meestal in de urine werden aangetroffen. De methode die werd ontwikkeld voor het meten van de afzonderlijke enantiomeren van baclofen, zal in de toekomst toepasbaar moeten worden gemaakt voor biologisch materiaal. Voor de $\gamma$-hydroxymetaboliet van baclofen lijkt geen rol van betekenis te zijn weggelegd bij mens en hond. Er werden aanwijzingen gevonden voor een lineair verband tussen de creatinineklaring en de renale klaring van onveranderd baclofen, ongeacht de isomere samenstelling. Gedetailleerd onderzoek met enantioselectieve analysemethodes is noodzakelijk voor het verder in kaart brengen van de farmacokinetiek van baclofen.

De voor dantroleen ontwikkelde analysemethode bleek voldoende gevoelig en specifiek te zijn voor het kwantitatief meten van onveranderd dantroleen en twee van zijn metabolieten. Hiermee konden concentraties in plasma, urine en gal worden gemeten. In tegenstelling tot de bevindingen bij de hond werden bij de mens echter geen meetbare concentraties in de gal aangetroffen. Een metaboliet die na verbreking van de azomethinebinding kan worden gevormd, bleek ook niet bij te dragen aan de verre van volledige massabalans van dit geneesmiddel bij de mens. Er werden aanwijzingen gevonden dat door hydroxylering van dantroleen de halfwaardetijd wordt bekort. Toekomstig onderzoek zal zich moeten toespitsen op het metabolisme van dit geneesmiddel.

De klinische bevindingen geven aan hoe men te werk kan gaan bij onderzoek naar het verband tussen plasmaconcentratie en effect bij patienten met cerebral palsy, met name bij een eveneens aanwezige geestelijke handicap. Andere antispastica, zoals het meer recent beschikbaar gekomen tizanidine, kunnen in principe ook met een dergelijke proefopzet worden geevalueerd. Gezien het racemisch karakter van klinisch toegepast baclofen en de nog verre van volledige massabalans van dantroleen is het niet verwonderlijk dat vooralsnog geen duidelijke uitspraken kunnen worden gedaan over concentratie-effect relaties. Een bijkomend gevolg van de verkennende klinische studie is geweest, dat men in het instituut waar dit onderzoek plaatsvond, minder antispastica is gaan gebruiken.

## Curriculum vitae

The author was born in Amsterdam, the Netherlands, on July 22nd 1948. Shortly after her third birthday, she drew her first 'graph' (Fig 1). A year later in kindergarten she met one of her future "paranimfen". While in primary school she used to play in the neighbourhood pharmacy From 1960-1963 she attended the Hervormd Lyceum in Amsterdam, and from 1963-1966 the Karel van Mander Lyceum in Haarlem. During her final school year, she joined a school project at Duphar in Weesp, where she first encountered chromatography, a subject she then chose as an elective in her biology finals. She graduated from secondary school in 1966 (gymnasium-β). While employed as au pair in London in 1967 she obtained the certificate· English for Foreigners of the Royal Society of Arts. In the same year she worked for several months at the Research and Development Division of Gist-Brocades, at that time located in Haarlem, but presently in Delft.

In September 1967 she started her pharmaceutical education at the State University of Groningen. She passed the "kandidaatsexamen farmacie" cum laude in 1970, followed in 1974 by the "doctoraalexamen farmacie" with medical microbiology as elected subsidiary subject. From 1971-1973 she also worked as a teaching-assistant in animal physiology laboratory courses. In 1975 she graduated as a pharmacist. In the same year she accepted a position at the Royal Dutch Association for the advancement of Pharmacy (KNMP) in the Hague. When she resigned in 1979, she was head of the Drug Information Centre of the KNMP. To continue her pharmaceutical education, she spent a year at the School of Pharmacy of the University of Michigan in Ann Arbor (MI, USA), where she attended the Pharm D programme. In October 1980 she was employed by the University Hospital Nijmegen (St Radboud) in the Department of Clinical Pharmacy. She was registered as a hospital pharmacist in 1986. From 1980 1987 she was also charged with the supervision over the pharmacy of Huize Boldershof, an institution for the mentally retarded, in Druten. During that time she started the investigations into the pharmacokinetics of drugs effective in the management of spasticity.

She is the (co)-author of the following publications:

1. Cox HLM, Wuis EW. Antipyretische analgetica voor jonge kinderen (Antipyretic analgesics for infants). Pharm Weekbl 1979; 114: 1355-67.

*2. Wuis EW, Grutters ACLM, Vree TB, Van der Kleijn E. Simultaneous determination of dantrolene and its metabolites, 5-hydroxydantrolene and nitro-reduced acetylated dantrolene (F490), in plasma and urine of man and dog by high-performance liquid chromatography. J Chromatogr 1982; 231: 401-9.

3. Vree TB, Hekster YA, Oosterbaan MJM, Wuis EW. De gedragingen van ethanol en andere alcoholen in het lichaam van de mens [The elimination of ethanol and other alcohols from the human body]. Tijdschr Alc Drugs 1983; 9: 38-46.

4. Vree TB, Hekster YA, Oosterbaan MJM, Wuis EW, Nouws JFM. Enkele kinetische criteria voor de keuze van geneesmiddelen gedemonstreerd met behulp van vier sulfonamide structuuranaloga [Some kinetic criteria in drug choice illustrated by means of four sulfonamide structure analogues]. J Drug Ther Res 1983; 8: 1917-20.

*5. Wuis EW, Dirks RJM, Vree TB, Van der Kleijn E. High-performance liquid chromatographic analysis of baclofen in plasma and urine of man after precolumn extraction and derivatization with o-phthaldialdehyde. J Chromatogr 1985; 337: 341-50.

6. Driessen JJ, Wuis EW, Gielen MJM. Prolonged vecuronium blockade in a patient receiving orally administered dantrolene. Anesthesiol 1985; 62: 523-4.

7. Wuis EW, Hekster YA, Zuidgeest LBJ, De Goede W, Hommes O, Keyser A. Patterns in drug utilization for antiepileptic drugs in a university hospital. J Soc Admin Pharm 1985; 3: 59-63.

*8. Wuis EW, Driessen JJ, Evers J, Vree TB, Van der Kleijn E. Dantrolene plasma and urine concentrations after oral pretreatment for malignant hyperthermia: report of a case. Eur J Anaesthesiol 1986; 3: 219-23.

9. Wuis EW. Tizanidine (Sirdalud®). Pharm Weekbl 1986; 12: 1114-5.

*10. Wuis EW, Beneken Kolmer EWJ, Van Beijsterveldt LEC, Burgers RCM, Vree TB, Van der Kleijn E. Enantioselective high-performance liquid chromatographic determination of baclofen after derivatization with a chiral adduct of o-phthaldialdehyde. J Chromatogr 1987; 415: 419-22.

*11. Wuis EW, Van Beijsterveldt LEC, Dirks RJM, Vree TB, Van Der Kleijn E. Rapid simultaneous determination of baclofen and its γ-hydroxy metabolite in urine by high-performance liquid chromatography with ultraviolet detection. J Chromatogr 1987; 420: 212-6.

*12. Wuis EW. Spasticity and drug therapy Pharm Weekbl [Sci] 1987; 9 249-60.

13. Ariens EJ, Wuis EW. Bias in pharmacokinetics and clinical pharmacology. Clin Pharmacol Ther 1987; 42: 361-3.

14. Duchateau AMJA, Wuis EW. Kunnen we stereoisomeren blijven negeren? [Can we continue to ignore stereoisomerism?] Pharm Weekbl 1987; 122: 541-3.

15. Termond EFS, Hekster YA, Wuis EW, Campschreur T, Vree TB. Geneesmiddeltherapie begeleiding; evaluatie van een begeleiding in een academisch ziekenhuis [A drug therapy monitoring evaluation in an academic hospital]. J Drug Res 1987; 12· 172-6.

16. Ariens EJ, Wuis EW, Veringa EJ. Stereoselectivity of bioactive xenobiotics. A pre-Pasteur attitude in medicinal chemistry, pharmacokinetics and clinical pharmacology. Biochem Pharmacol 1988; 37: 9-18.

*17. Wuis EW, Rijntjes NVM, Van der Kleijn E. Whole-body autoradiography of $^{14}$C-dantrolene in the marmoset monkey. Pharmacol Toxicol 1989; 64: 156-8.

*18. Wuis EW, Dirks MJM, Termond EFS, Vree TB, Van der Kleijn E. Comparison of the pharmacokinetics of rac-baclofen and its (-)-(R)- and (+)-(S)-enantiomers in dogs. Int J Clin Pharmacol Res 1989; 10: 239-46.

*19. Wuis EW, Dirks MJM, Termond EFS, Vree TB, Van der Kleijn E. Plasma and urinary kinetics of oral baclofen in healthy subjects. Eur J Clin Pharmacol 1989; 37: 181-4.

*20. Wuis EW, Janssen MGA, Vree TB, Van der Kleijn E. Determination of a dantrolene metabolite, 5-(p-nitrophenyl)-2-furoic acid, in plasma and urine by high-performance liquid chromatography. J Chromatogr 1990; 526: 575-80.

*21. Wuis EW, Dirks MJM, Vree TB, Van der Kleijn E. Pharmacokinetics of baclofen in spastic patients receiving multiple oral doses. Pharm Weekbl [Sci] 1990; 12: 71-4.

*22. Wuis EW, Vree TB, Van der Kleijn E. Pharmacokinetics of intravenously administered dantrolene and its 5-hydroxy metabolite in dogs. Int J Clin Pharmcol Res, in press.

23. Vree TB, Baars AM, Wuis EW. De bepaling en farmacokinetiek van de enantiomeren van oxazepam en temazepam met hun glucuronide-conjugaten [HPLC analysis and pharmacokinetics of the enantiomers of R,S-oxazepam and R,S-temazepam with their corresponding

glucuronide conjugates in urine and plasma of man]. Acta Neuropsych, in press.

*24. Wuis EW, Blankesteijn RS, Rotteveel JJ, Vingerhoets HM, Van Loon YEJ, Verhey HEG, Zweers-Ewalts AMH, Van der Kleijn E. Effects and plasma concentrations of baclofen and dantrolene in mentally retarded patients with cerebral palsy: a preliminary report. Arch Phys Med Rehabil, submitted.

25. Wuis EW, Hendriks GJH, Vree TB, Van der Kleijn E. Pharmacokinetics of oral and intravenous dantrolene in healthy subjects, in preparation.

26. Wuis EW, Botterblom MHA, Vree TB, Gielen MJM, Van der Kleijn E. Pharmacokinetics of dantrolene after oral pretreatment in patients at risk of malignant hyperthermia, in preparation.

*included as chapters in this thesis.

Contributions to scientific conferences have resulted in the following abstracts:

1. Wuis EW, Bosman FC. Compilation of a computerized pharmaco-therapeutical handbook. FIP 77 Abstracts, 37th International Congress of Pharmaceutical Sciences, The Hague, 1977, p 188.

2. Wuis EW, Hendriks HJ, Vree TB, Van der Kleijn E. Pharmacokinetics of dantrolene in man after oral administration. Abstracts II International Conference on Clinical Pharmacology and Therapeutics, Washington DC, 1983, p 12.

3. Wuis EW, Van Loon YEJ. Therapeutic team management in spasticity. Abstracts 13th European Symposium on Clinical Pharmacy, Bournemouth, 1984, p 12.

4. Van der Kleijn E, Rosingh E, Hekster YA, Vree TB, Wuis EW, Zuidgeest LBJ, Arens A, Kwa HY, Van der Lei J, Hasman A. Computer supported diagnostic and therapeutic decisions [Abstract]. Pharm Weekbl [Sci] 1984; 6: 271.

5. Hekster YA, Friesen WT, Van der Kleijn E, Mens W, Vree TB, Wintermans R, Wuis EW, Zuidgeest LBJ. Therapeutic audit: the balance between drug utilization and the quality of care [Abstract]. Pharm Weekbl [Sci] 1984; 6: 272.

6. Wuis EW, Beneken Kolmer EWJ, Van Beijsterveldt LEC, Burgers RCM, Vree TB, Van Der Kleijn E. Enantioselective high-performance liquid chromatographic analysis of baclofen after derivatization with a chiral adduct of o-phthaldialdehyde [Abstract]. Pharm Weekbl [Sci] 1986; 8: 318.

7. Wuis EW, Dirks MJM, Termond EFS, Vree TB, Van der Kleijn E. Pharmacokinetics of racemic baclofen and its separate enantiomers in dogs [Abstract]. New Trends Clin Neuropharmacol 1988; 2: 178.

8. Wuis EW, Termond EFS, Vree TB, Van der Kleijn E, Baarsma JP. Pharmacokinetics of baclofen in man. Intra- and intersubject variability in some model-independent parameters. In: Marsden D, ed. Treating spasticity: pharmacological advances. Bern: Hans Huber Publishers, 1989; 69-72.

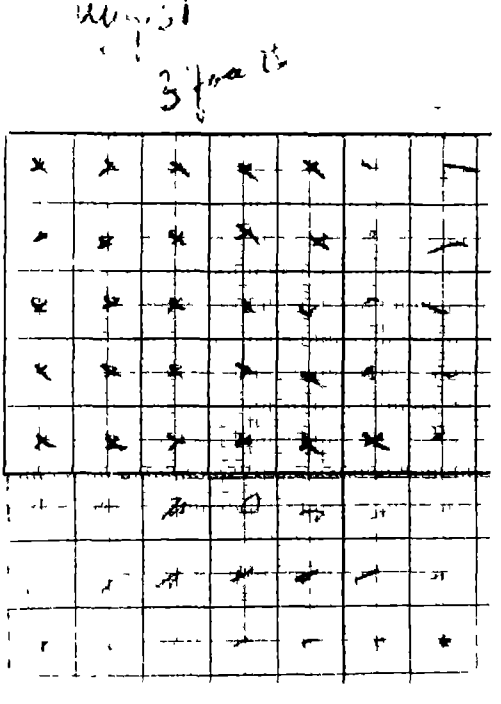

FIGURE 1
First 'graph'

**STELLINGEN**

1. Aangezien de aanduiding R of S ter onderscheiding van de enantiomeren van een verbinding uitsluitend wordt toegekend op grond van afspraken betreffende de prioriteit van de substituenten aan het chirale centrum, kan men er niet van uitgaan dat een bepaalde configuratie van een geneesmiddel een metaboliet met dezelfde aanduiding oplevert.

2. Het verdient aanbeveling om, in analogie met het Angelsaksische taalgebruik, geneesmiddelen die bij de behandeling van spasticiteit worden gebruikt antispastica te noemen.

   - dit proefschrift

3. Resorptieverschillen vormen niet de enige verklaring voor de farmacokinetische verschillen tussen de enantiomeren van baclofen.

   - Krauss D, Dissertation, Frankfurt, 1988

   - dit proefschrift

4. De verre van volledige massabalans van dantroleen valt niet te verklaren uit de algemeen aanvaarde slechte resorptie.

5. Het Geneesmiddelvergoedingssysteem dat de Staatssecretaris van WVC wil invoeren zal niet noemenswaardig bijdragen aan een beheersing van de kosten van het gebruik van antispastica.

   - brief dd 13 november 1990 aan de Voorzitter van de Tweede Kamer der Staten-Generaal

6. Homeopathische middelen werken niet maar helpen soms wel, antispastica werken wel maar helpen soms niet.

7. Het is opmerkelijk dat bij de ziekenhuisopname van patienten niet standaard het lichaamsgewicht wordt gemeten.

8. Bij knoflook-overgevoeligheid is 'buiten de deur eten' steeds weer een hachelijk avontuur, dat al begint bij het (gratis) voorafje.

9. Wie glasafval per fiets vervoert, is een milieuvriendelijke optimist gezien de onvermijdelijke scherven in de onmiddellijke omgeving van glasbakken.

Nijmegen, 15 januari 1991 Eveline W. Wuis