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Renal Tubular Excretion of the N_4-Acetyl Metabolites of Sulphasomidine and Sulphadimethoxine in the Dog

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Abstract—To investigate whether dogs are able to excrete acetylated drugs by active transport, the plasma kinetics and renal excretion of the N_4-acetyl metabolites of sulphasomidine and sulphadimethoxine were studied in the beagle dog after a rapid intravenous bolus injection. Two doses of N_4-acetylsulphasomidine (1050 and 105 mg) and one dose of N_4-acetylsulphadimethoxine (472 mg) were administered on separate occasions. The renal clearance (CL_R) was as follows: N_4-acetylsulphasomidine (1050 mg) 34 mL min^{-1}; N_4-acetylsulphasomidine (105 mg) 28 mL min^{-1}; and N_4-acetylsulphadimethoxine (472 mg) 24 mL min^{-1}. CL_R was higher than expected on the basis of the measured glomerular filtration rate, indicating that the N_4-acetyl metabolites may be excreted by the renal tubules by active tubular transport. Saturation of the excretion process of N_4-acetylsulphasomidine occurred with a transport maximum of 930 ± 190 μg min^{-1} and a Michaelis-Menten constant of 37 ± 10 μg mL^{-1}. It may be concluded that the dog renal organic anion transport system is able to secrete acetylated sulphonamides.

Sulphonamides comprise a structurally related group of antibacterial agents with similar chemotherapeutic actions (Vree & Hekster 1987). Their metabolism and renal handling, however, differ widely and depend on the chemical structure of the N_1-substituent, the species and metabolic capacity (Bridges et al 1968; Vree & Hekster 1987).

Dogs appear to be unable to acetylate sulphonamides, since no N_4-acetylated derivatives of xenobiotics have been detected (Vree et al 1983). However, other species including sheep, goat, rabbit, monkey, and man are able to acetylate these antibacterial agents (Smith & Williams 1948; Bridges et al 1968, 1969). In man, all N_4-acetylsulphonamides that have been studied so far, are excreted by an active tubular transport mechanism (Vree & Hekster 1987). Co-administration of probenecid lowers this excretion, indicating transport via the organic anion transport mechanism (Vree et al 1979). The renal handling of the parent compounds in man is different; sulphasomidine is excreted by glomerular filtration and active tubular transport, while sulphadimethoxine undergoes net reabsorption.

An anionic transport mechanism in the renal tubule probably has the function of excreting endogenous waste compounds and therefore it must be able to recognize functional groups for binding. The capacity for active tubular excretion is developed by the kidney tubule during the first years of life (Taggart 1958; Nash & Edelmann 1973; Hewitt & Hook 1976; Frenzel et al 1977; Hook & Hewitt 1977; Braunlich 1981; Appenroth et al 1981). If the stimulation and development of active tubular transport by certain substrates does not take place because certain functional groups are not presented to the organism, this may result in the absence of a specific transport mechanism.

The aim of this study was to investigate whether dogs are able to excrete N_4-acetylsulphonamides via an active tubular transport mechanism. For this purpose the renal clearance of two N_4-acetylsulphonamides was determined; N_4-acetylsulphasomidine was selected because sulphasomidine itself is secreted and N_4-acetylsulphadimethoxine was selected because sulphadimethoxine shows net tubular reabsorption.

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Materials and Methods

Dogs

Two male beagle dogs were obtained from the Central Animal Laboratory of the University of Nijmegen.

Drugs

N_4-Acetylsulphasomidine and N_4-acetylsulphadimethoxine were synthesized as described by Vree et al (1979). N_4-Acetylsulphasomidine contained 5% sulphasomidine (w/w) as determined by high-performance liquid chromatography (HPLC). N_4-Acetylsulphadimethoxine was 100% pure. Sodium pentobarbitone was obtained from Apharma (Arnhem, The Netherlands), atropine sulphate and mannitol from O.P.G. (Utrecht, The Netherlands), [^{14}C]inulin (2·03 Ci $mmol^{-1}$) from Amersham (Buckinghamshire, UK). All other chemicals were purchased from Merck (Darmstadt, Germany).

Clearance experiments

The clearance experiments were performed as described in detail previously (Russel et al 1987). The dogs were anaesthetized intravenously with sodium pentobarbitone (30 mg kg^{-1}) combined with atropine sulphate (0·5%, 1 mL). For blood sampling and drug administration the cephalic veins were cannulated (Braunule T, length 50 mm, 1·0 o.d. × 1·5 i.d. mm, Braun, Melsungen, Germany). An infusion of mannitol (5%, 2 mL min^{-1}) was administered to obtain a sufficiently high urine flow. The N_4-acetylsulphonamides were administered intravenously as sterile solutions (60 mg mL^{-1}) in diluted NaOH (pH 9). Blood samples (7 mL) were taken at regular intervals into heparinized tubes and plasma

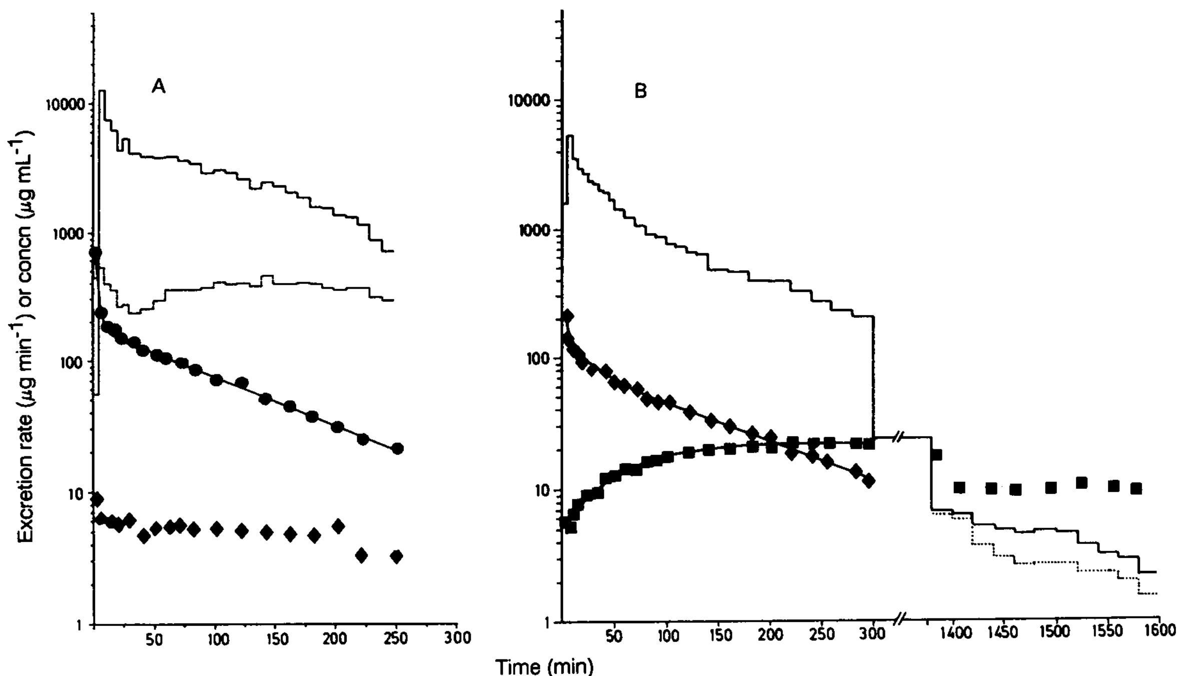

FIG. 1. Renal excretion rate and plasma concentrations of (A) injected drug following intravenous injection of 1050 mg N_4-acetylsulphasomidine (———, ●) and 50 mg of its metabolite sulphasomidine (——, ◆), and (B) injected drug and its metabolite, sulphadimethoxine (......, ■) following intravenous injection of 472 mg N_4-acetylsulphadimethoxine (——, ◆).

was separated by centrifugation (6 min, 2000 *g*). Urine was collected quantitatively using a double-walled urinary catheter by rinsing the bladder with 10 mL 0·9% NaCl (saline). The plasma and urine samples were stored at −20°C until analysis. The experiment with N_4-acetylsulphadimethoxine was carried out over two days because of the expected long half-life of N_4-acetylsulphasomidine, and the dog was anaesthetized during both days.

The glomerular filtration rate (GFR) was determined by steady-state [^{14}C]inulin clearance each day at the end of the experiment (Russel et al 1987). An infusion of [^{14}C]inulin (0·5 μCi mL^{-1}) in saline was given (10 mL h^{-1}) with a concomitant injection of 5 mL of this solution. Plasma and urine samples were taken every 15 min during a 45-min period.

Analytical methods

Plasma and urine samples were analysed for components of interest by HPLC (Vree et al 1979). The following equipment was used: a Spectra-Physics SP 8810 pump with a stainless steel column packed with Spherisorb 5 ODS (25 cm long, 4·6 mm i.d.) for N_4-acetylsulphasomidine and sulphasomidine, and Chromspher C_8 (10 cm long, 4·6 mm i.d.) for N_4-acetylsulphadimethoxine and sulphadimethoxine (Chrompack, Middelburg, The Netherlands) and a variable wavelength detector (Spectra-Physics, model 770) set at 272 nm. The mobile phase used for N_4-acetylsulphasomidine and sulphasomidine was a mixture containing 1·14 mM acetic acid, 17·9 mM sodium acetate and 8% acetonitrile (v/v). For the determination of N_4-acetylsulphadimethoxine and sulphadimethoxine the following mobile phase was used: 5 mM acetic acid, 5 mM phosphoric acid, 1 mM trimethylammoniumchloride in aqua bidest with 13% methanol (v/v), 5% acetonitrile (v/v) and 5% dimethylformamide (v/v).

The detection limits for the compounds were as follows: for N_4-acetylsulphasomidine and sulphasomidine in plasma 0·3 μg mL^{-1} and in urine 5 μg mL^{-1}; for N_4-acetylsulphadimethoxine and sulphadimethoxine in plasma 0·1 μg mL^{-1} and in urine 0·5 μg mL^{-1}. The accuracy and precision were greater than 5%.

Protein binding

Protein binding was determined by ultrafiltration using an Amicon Micropartition System (MPS-1) equipped with YMT membranes (Amicon, Oosterhout, The Netherlands) and the free fraction of drug in plasma (f_u) was calculated.

Pharmacokinetic analysis

Plasma and urine data were analysed separately by means of the nonlinear regression program NONLIN (Metzler et al 1974). Basic pharmacokinetic parameters (clearance and distribution volume) were calculated model-independently applying the statistical moments theory, using standard equations (Yamaoka et al 1978; Benet & Galeazzi 1979; Gibaldi & Perrier 1982). Half-life ($t\frac{1}{2}$) of the drug in plasma was determined from the terminal phase. The area under the plasma curve (AUC) and the area under the renal excretion rate curve were determined by integration after extrapolation of the terminal phase of the curve to infinity. The amount excreted into urine in infinite time (D_{ur}) was obtained from the area under the renal excretion rate–time profile. Apparent renal clearance (CL_R) of the N_4-acetyl metabolites was estimated by dividing the area under the renal excretion

Table 1. Pharmacokinetic and experimental parameters characterizing the renal handling of N_4-acetylsulphasomidine (NSS) and N_4-acetylsulphadimethoxine (NSDM).

Compound	NSS	NSS	NSDM
Dog	1	1	2
Weight (kg)	14·2	13·8	14·5
Dose (mg)	1050	105	472
Urine pH[a]	7·88±0·18	7·29±1·10	7·36±0·36
Urine flow[a] (mL min^{-1})	1·3±0·4	1·0±0·2	0·9±0·3
GFR[b] (mL min^{-1})	54±1	34±2	27±5
f_u[c]	0·30±0·03	0·23±0·07	0·20±0·02
D_{ur} (% dose)	86	71	61
$t_{\frac{1}{2}}$ (min)	79	76	100
CL (mL min^{-1})	45	40	32
CL_R (mL min^{-1})	34	28	24
V_{ss} (L)	4·4	4·0	4·2

Data are expressed as means ± s.d. GFR: glomerular filtration rate, f_u: free fraction of drug in plasma, D_{ur}: amount excreted into urine in infinite time, $t_{\frac{1}{2}}$: half-life of the terminal phase of drug in plasma, CL: total plasma clearance, CL_R: renal clearance, V_{ss}: distribution volume at steady-state. [a] Average of all urine samples (n = 25). [b] Average of steady-state inulin clearance (n = 3). [c] Average of determination of protein binding (n = 6).

rate–time profile curve by the AUC. CL_R of sulphasomidine and sulphadimethoxine was calculated by dividing the renal excretion rate of each urine collection interval by its corresponding plasma concentration. These data were averaged and expressed as mean ± s.d.

The tubular titration curves of the N_4-acetyl metabolites were constructed by plotting renal excretion rate vs plasma concentration. The curve of N_4-acetylsulphasomidine was analysed according to Michaelis–Menten kinetics in combination with glomerular filtration:

$$\text{renal excretion rate} = (V_{max} \cdot C/(K_m + C)) + GFR \cdot f_u \cdot C$$

where V_{max} is transport maximum, K_m is Michaelis–Menten constant, C is plasma concentration, GFR is glomerular filtration rate, and f_u is fraction unbound of drug in plasma (Russel et al 1987).

Average values are expressed as means ± s.d. The statistical significance was determined with the Wilcoxon-signed rank test. A value of $P < 0·05$ was considered significant.

Results

N_4-Acetylsulphasomidine

Two doses of N_4-acetylsulphasomidine which contained 5% (w/w) sulphasomidine were administered to the same dog: 1050 and 105 mg. Fig. 1A shows the plasma concentration–time curves and renal excretion rate–time profiles of N_4-acetylsulphasomidine and sulphasomidine in the beagle dog after an intravenous dose of 1050 mg N_4-acetylsulphasomidine and 50 mg sulphasomidine. A similar kinetic profile was obtained with the low dose of 105 mg N_4-acetylsulphasomidine and 5 mg sulphasomidine. The pharmacokinetic parameters $t_{\frac{1}{2}}$, CL and V_{SS}, obtained with this low dose were not significantly different from those with the high dose ($P > 0·10$) (Table 1). From the plasma concentration–time and the renal excretion rate–time curves, the apparent renal clearance of sulphasomidine was estimated to be 69 ± 18 mL min^{-1}, indicating active secretion. The renal clearance of N_4-acetylsulphasomidine was 34 and 28 mL min^{-1} for the high and low dose, respectively (Table 1).

N_4-Acetylsulphadimethoxine

The plasma concentration and renal excretion rate vs time curve of an intravenous dose of 472 mg N_4-acetylsulphadimethoxine is also shown in Fig. 1B. The experiment was carried out over two days because of the expected long half-life of N_4-acetylsulphadimethoxine. The GFR was the same on both days. Although N_4-acetylsulphadimethoxine is deacetylated to sulphadimethoxine to some extent, only a small percentage of the dose (1·7%) was excreted as sulphadimethoxine within 27 h. Excretion of sulphadimethoxine in the urine could not be detected until the second day. From the urinary excretion rate and the plasma concentration of sulphadimethoxine on the second day the renal clearance was calculated to be 0·3 ± 0·1 mL min^{-1}. The clearance by glomerular filtration of the free fraction of the drug in plasma ($f_u = 0·13 ± 0·01$), was 3·5 mL min^{-1}, indicating that sulphadimethoxine is extensively reabsorbed in the dog. The renal clearance of N_4-acetylsulphadimethoxine was 24 mL min^{-1}, signifying active tubular excretion.

Renal excretion

The renal excretion of both N_4-acetylsulphasomidine and N_4-acetylsulphadimethoxine is determined by glomerular filtration and tubular excretion, as can be seen from the tubular titration curves (Fig. 2). The renal excretion rate is higher than would be expected from the glomerular filtration rate alone, which suggests that the N_4-acetyl metabolites are subject to active transport. In the case of N_4-acetylsulphasomidine, transport is saturated at the highest plasma concentrations. The titration curve then parallels the glomer-

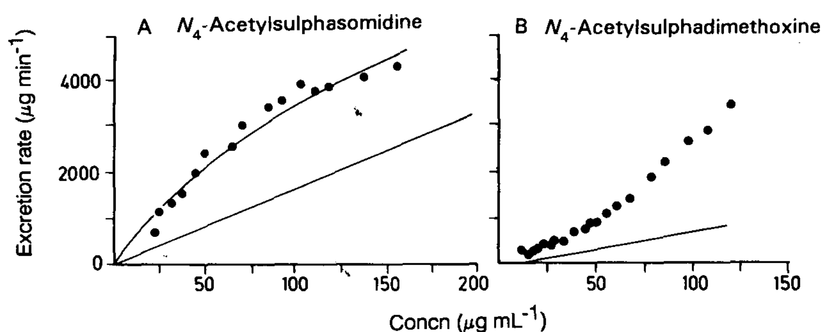

FIG. 2. Relationship between the renal excretion rate and the plasma concentration of (A) N_4-acetylsulphasomidine (1050 mg, i.v.) and (B) N_4-acetylsulphadimethoxine (472 mg, i.v.). The straight line represents the clearance of the free fraction of N_4-acetylsulphonamides by glomerular filtration.

ular filtration. The tubular transport maximum is 930 ± 190 μg min^{-1} and the Michaelis–Menten constant 37 ± 10 μg mL^{-1}.

Discussion

The dog is apparently unable to metabolize sulphonamides by means of acetylation. Therefore, it is conceivable that the dog renal organic anion transport system does not recognize acetylated sulphonamides. However, this does not seem to be the case, as the N_4-acetylated metabolites of the two sulphonamides studied were actively excreted by the dog. There is no relation with the renal handling of the sulphonamide itself, as sulphasomidine is actively excreted ($CL_R = 69 \pm 18$ mL min^{-1}), while sulphadimethoxine is reabsorbed ($CL_R = 0{\cdot}3 \pm 0{\cdot}1$ mL min^{-1}).

The renal excretion rate vs plasma concentration plots of N_4-acetylsulphasomidine and N_4-acetylsulphadimethoxine were different: the excretion of N_4-acetylsulphasomidine was saturated at a plasma concentration of approximately 120 μg mL^{-1} (thus not observed with the low dose), while the excretion of N_4-acetylsulphadimethoxine showed no signs of saturation at the same plasma concentration. N_4-Acetylsulphadimethoxine probably has a higher Michaelis–Menten constant (K_m) than N_4-acetylsulphasomidine, as a result of which saturation of excretion occurs at higher plasma concentrations. Since the saturation of the renal clearance of N_4-acetylsulphasomidine occurred only in the initial phase of the plasma curve of the high dose, the overall plasma kinetics were not influenced in comparison with the low dose.

The saturation of the active tubular excretion of N_4-acetylsulphasomidine at the highest plasma concentrations may be enlarged by the small amount (5%) of sulphasomidine that was present as co-medication. If pure N_4-acetylsulphasomidine were administered, sulphasomidine would also be present as a metabolite, though in slightly lower concentrations. In this respect, interaction at the level of active tubular excretion cannot be ruled out in the dog. Interaction studies, therefore, should be carried out with increasing doses of sulphasomidine in order to demonstrate that both sulphasomidine and N_4-acetylsulphasomidine have their own K_m and transport maximum of the organic anion carrier.

The results of this investigation have the following impact on our understanding of active tubular secretion. The N_4-acetylphenylamino group does not play a role in the development of the anion transport mechanism in the dog kidney tubule, as the tubule never encounters such a group, nevertheless, it is able to secrete this functional group. Thus drugs and drug metabolites are recognized by the anion transport mechanism and excreted, even when these drugs contain non-endogenous functional groups.

In summary, both N_4-acetylsulphasomidine and N_4-acetylsulphadimethoxine are actively excreted by the dog, indicating that the dog organic anion transport system is capable of recognizing and actively transporting N_4-acetylsulphonamides.

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