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RENAL EXCRETION OF N^4-ACETYL SULPHANILAMIDE AND N^4-ACETYL SULPHADIMIDINE IN GOATS

By

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JØRGENSEN, SIGRID TUE, POUL NIELSEN and FOLKE RASMUSSEN: *Renal excretion of N^4-acetyl sulphanilamide and N^4-acetyl sulphadimidine in goats.* Acta vet. scand. 1974, *15*, 188—197. — The renal excretion of N^4-acetyl sulphanilamide and N^4-acetyl sulphadimidine was studied in 19 experiments with 6 goats during continuous intravenous administration of the 2 sulphonamide derivatives. Deacetylation of both compounds takes place to a small extent only. Further it is shown that both sulphonamide derivatives are bound to plasma proteins to a greater extent than sulphanilamide and sulphadimidine. The excretion of the N^4-acetylated sulphonamides is compared with the renal excretion of creatinine. The non-protein-bound fraction of the 2 N^4-acetylated sulphonamides is excreted by filtration and active tubular secretion. The renal clearances of the acetyl derivatives are higher than those of the parent compounds.

renal excretion; sulphonamides; N^4-acetyl sulphanilamide; N^4-acetyl sulphadimidine; protein-binding; deacetylation.

In experiments concerning the renal excretion of sulphonamides it has been reported that the renal clearance is considerably higher for the endogenously synthesized N^4-acetyl derivatives than for the parent compounds. For instance this has been shown for sulphanilamide in man (*Frisk* 1943, *Loomis et al.* 1944), dogs (*Arita et al.* 1972), and goats (*Jørgensen & Rasmussen* 1972); for sulphadimidine (4,6-dimethyl-2-sulphanilamido-pyrimidine) in man (*Volini et al.* 1945, *Fischer* 1972), pigs (*Dalgaard-Mikkelsen & Poulsen* 1956), goats (*Jørgensen & Rasmussen*), and cows (*Nielsen* 1973); for sulphadoxine (5,6-dimethoxy-4-sulphanilamido-pyrimidine) in man (*Madsen & Iversen* 1963, *Reber et al.*

1963, *Böhni et al.* 1969) and for sulphamethoxazole (5-methyl-3-sulphanilamido-isoxazole) in man (*Reber et al.*).

The purpose of the present work was to investigate the renal excretion in goats of exogenously synthesized N^4-acetyl sulphanilamide and N^4-acetyl sulphadimidine after intravenous administration to the animals.

MATERIALS AND METHODS

Six goats weighing from 30 to 45 kg were used in 19 experiments. The N^4-acetyl sulphonamides were dissolved in distilled water by heating and simultaneous addition of 5 N-NaOH. The solutions were then administered to the goats by an initial intravenous injection followed by a continuous infusion in order to keep the plasma concentration at a constant level. Analysis showed that the solutions administered to the goats contained exclusively N^4-acetyl sulphonamide. The initial dose of N^4-acetyl sulphanilamide and N^4-acetyl sulphadimidine was 15—70 mg/kg b.wt. and 7—35 mg/kg b.wt., respectively, while the maintenance dose varied between 25 and 140 mg/kg b.wt. for both compounds.

The experiments with N^4-acetyl sulphonamide alone comprised 5 experimental periods, each of 30 min. duration. The experiments with both N^4-acetyl sulphonamide and diodone comprised 6 periods. During the last 3 periods diodone (Diodonum NFN) and N^4-acetyl sulphonamide were administered simultaneously to the goats. The diodone was dissolved in distilled water and administered to the goats in the same way as the N^4-acetyl sulphonamide in order to ensure a constant plasma concentration. The initial and the maintenance doses of diodone were 100 mg/kg b.wt. and 450—600 mg/kg b.wt., respectively.

Blood samples were collected in heparinized vials 10 min. after the beginning of every experimental period. Urine was collected quantitatively by means of a balloon catheter (Rüsch no. 14, 30 ml) which was placed in the bladder.

The pH-values of the blood and urine samples were measured potentiometrically with micro glass electrode (Radiometer) at 37°C immediately after finishing each experiment. Protein-binding was determined by ultrafiltration through cellophane membrane (*Poulsen* 1956). The concentration of free sulphonamide in the plasma and urine samples was determined by the

method of *Bratton & Marshall* (1939). The concentration of total sulphonamide was determined in the same samples after hydrolysis with hydrochloric acid. The concentration of N^4-acetylated sulphonamide was calculated as being the difference between the concentration of total and that of free sulphonamide.

The renal clearance of sulphonamide is compared with the renal clearance of creatinine which expresses the filtration clearance in goats as shown by *Jørgensen & Rasmussen* (1972).

RESULTS

N^4-acetyl sulphanilamide

The N^4-acetyl sulphanilamide was deacetylated to a small extent in the goats. The amount of free sulphanilamide varied between 9 and 14 % of the total sulphonamide in the blood plasma while only 3—6 % appeared in the urine samples.

From Table 1 can be seen that the clearance of N^4-acetyl sulphanilamide in blood plasma is higher than or equal to the clearance of creatinine (column 9). On an average 28 ± 1 % (m. ± s.e.m.) of the 4^4-acetyl sulphanilamide is bound to the plasma proteins, and the clearance of the non-protein-bound N^4-acetyl sulphanilamide is 1.3—2 times higher than the clearance of creatinine (last column). The clearance of N^4-acetyl sulphanilamide is independent of its concentration in the plasma in an interval from 15 to 87 µg N^4-acetyl sulphanilamide per ml (Table 1).

In 1 experiment the renal excretion of N^4-acetyl sulphanilamide was determined during loading with diodone, and the results from this experiment are shown in Table 2. It appears that diodone loading distinctly reduces the clearance of N^4-acetyl sulphanilamide. Calculation of the ratio between the clearance of non-protein-bound N^4-acetyl sulphanilamide and the clearance of creatinine shows that the ratio was about 2 before loading with diodone while it was reduced to about 1 during the loading.

N^4-acetyl sulphadimidine

From 6 to 13 % of the total contents of sulphonamide in the blood was found to be present in its free form while the corresponding value was 3—6 % in the urine samples.

Table 1. Clearance of creatinine and N^4-acetyl sulphanilamide.

Goat no.	Urine flow ml/min. min.-max.	Urine pH min.-max.	N^4-acetylated sulphonamide in blood plasma µg/ml	N^4-acetylated sulphonamide in ultra-filtrate of blood plasma µg/ml	Clearance ml/min./10 kg b.wt. N^4-acetylated sulphonamide in blood plasma	Clearance ml/min./10 kg b.wt. N^4-acetylated sulphonamide in ultra-filtrate of blood plasma	Clearance ml/min./10 kg b.wt. crea-tinine	Ratio clearance of N^4-acetylated sulphonamide in blood plasma / clearance of creatinine	Ratio clearance of N^4-acetylated sulphonamide in ultrafiltrate of blood plasma / clearance of creatinine
45	4.4—8.7	7.9—8.0	14.8	11.8	32.8	42.4	20.4	1.6	2.1
45	0.4—0.7	8.4—8.5	21.6	15.1	32.8	46.9	25.5	1.3	1.9
48	1.3—3.4	7.9—8.3	22.2	16.8	28.0	37.1	28.8	1.0	1.3
47	2.9—6.3	7.9—8.0	23.2	17.4	24.6	32.8	19.8	1.2	1.7
47	0.7—0.8	8.4—8.5	24.7	17.9	25.6	35.5	19.3	1.3	1.8
46	1.5—2.9	7.9—8.3	25.0	20.6	35.3	42.8	21.9	1.6	2.0
40	0.4—1.0	8.5—8.5	25.6	17.0	17.6	26.6	16.5	1.1	1.6
48	0.6—3.5	7.5—8.3	43.6	31.5	35.4	49.0	31.8	1.1	1.5
40	1.9—2.2	8.2—8.3	50.2	36.7	22.3	30.5	23.9	0.9	1.3
45	1.5—2.0	8.4—8.5	73.0	46.6	29.9	46.8	21.3	1.4	2.2
46	1.9—6.1	7.9—8.3	76.9	51.8	36.2	53.8	27.7	1.3	1.9
47	1.9—6.7	7.8—8.3	86.2	61.1	19.8	27.9	19.2	1.0	1.5
47	1.7—2.0	8.2—8.2	86.6	57.9	23.4	35.0	21.7	1.1	1.6
m. ± s.e.m.					28 ± 2	39 ± 2	23 ± 1	1.2 ± 0.1	1.7 ± 0.1

Table 2. Clearance of N^4-acetyl sulphanilamide in goat no. 45 during diodone blockade.

Observation no.	Conc. of N^4-acetyl sulphonamide in blood plasma µg/ml	Clearance ml/min./10 kg b.wt.			Ratio clearance of N^4-acetyl sulphonamide in blood plasma / clearance of creatinine	Ratio clearance of N^4-acetyl sulphonamide in ultrafiltrate of blood plasma / clearance of creatinine
		N^4-acetyl sulphonamide in blood plasma	N^4-acetyl sulphonamide in ultrafiltrate of blood plasma	creatinine		
1	15.0	31.6	40.8	18.1	1.7	2.3
2	13.0	31.9	41.2	19.2	1.7	2.2
3	15.5	35.0	45.1	24.0	1.5	1.9
Diodone						
4	16.2	13.9	17.9	17.8	0.8	1.0
5	19.6	13.0	16.7	18.5	0.7	0.9
6	17.6	13.9	17.9	18.6	0.7	1.0

From Table 3, which shows the results from the experiments with N^4-acetyl sulphadimidine, can be seen that clearance of N^4-acetyl sulphadimidine is 1.3—2 times higher than clearance of creatinine (column 9). On an average 87 ± 2 % (m. ± s.e.m.) of N^4-acetyl sulphadimidine is bound to the plasma proteins. This high protein-binding leads to a clearance of non-protein-bound N^4-acetyl sulphadimidine 9—15 times higher than the creatinine clearance (last column). As can be seen from the table the clearance of N^4-acetyl sulphadimidine is independent of the plasma concentration in an interval from 19 to 71 µg N^4-acetyl sulphadimidine per ml.

In 2 experiments the renal clearance of N^4-acetyl sulphadimidine was determined during simultaneous loading with diodone, and these results are shown in Table 4. From the table appears that loading with diodone markedly (60—70 %) diminishes the clearance of N^4-acetyl sulphadimidine, although the ratio between clearance of non-protein-bound N^4-acetyl sulphadimidine and clearance of creatinine still is higher than 1.

DISCUSSION

Previous experiments have shown that sulphanilamide and sulphadimidine are acetylated in goats, and in the present work it is shown that the N^4-acetyl derivatives of these 2 sulphon-

Table 3. Clearance of creatinine and N^4-acetyl sulphadimidine.

Goat no.	Urine flow ml/min. min.-max.	Urine pH min.-max.	N^4-acetylated sulphonamide in blood plasma µg/ml	N^4-acetylated sulphonamide in ultra-filtrate of blood plasma µg/ml	Clearance ml/min./10 kg b.wt. N^4-acetylated sulphonamide in blood plasma	Clearance ml/min./10 kg b.wt. N^4-acetylated sulphonamide in ultra-filtrate of blood plasma	Clearance ml/min./10 kg b.wt. creatinine	Ratio clearance of N^4-acetylated sulphonamide in blood plasma / clearance of creatinine	Ratio clearance of N^4-acetylated sulphonamide in ultrafiltrate of blood plasma / clearance of creatinine
43	1.2—1.7	7.5—7.8	18.6	2.8	49.3	330.7	35.9	1.4	9.2
47	1.4—3.7	6.7—6.7	20.2	2.3	31.2	271.6	27.3	1.5	10.0
47	1.1—3.3	7.2—7.6	24.0	3.5	44.0	301.0	20.7	2.1	14.5
40	2.7—4.0	7.6—8.0	40.7	3.7	43.1	468.0	32.2	1.3	14.5
43	1.6—1.8	7.9—8.0	58.3	5.1	44.9	510.2	34.2	1.3	15.0
47	1.7—2.6	6.9—8.1	71.4	13.6	44.0	231.5	21.3	2.1	10.9
m. ± s.e.m.					43 ± 3	352 ± 46	29 ± 3	1.6 ± 0.2	12.4 ± 1.1

Table 4. Clearance of N^4-acetyl sulphadimidine in 2 goats during diodone blockade.

Goat no.	Observation no.	Conc. of N^4-acetyl sulphonamide in blood plasma µg/ml	Clearance ml/min./10 kg b.wt.			Ratio clearance of N^4-acetyl sulphonamide in blood plasma / clearance of creatinine	Ratio clearance of N^4-acetyl sulphonamide in ultrafiltrate of blood plasma / clearance of creatinine
			N^4-acetyl sulphonamide in blood plasma	N^4-acetyl sulphonamide in ultrafiltrate of blood plasma	creatinine		
43	1	20.7	47.2	316.8	33.3	1.41	9.5
	2	18.4	48.9	328.2	35.0	1.39	9.4
	3	16.6	51.7	347.0	39.4	1.31	8.8
	Diodone						
	4	35.6	14.2	95.3	30.2	0.47	3.2
	5	36.6	14.3	96.0	25.0	0.57	3.8
	6	36.3	14.3	96.0	23.4	0.61	4.1
47	1	22.5	31.5	273.9	27.3	1.15	10.0
	2	19.7	31.5	269.6	28.9	1.08	9.3
	3	18.4	31.2	271.3	25.6	1.21	10.6
	Diodone						
	4	28.8	8.2	71.3	26.6	0.30	2.7
	5	30.9	8.8	76.5	25.4	0.34	3.0
	6	31.9	9.2	80.0	23.8	0.38	3.4

amides are also deacetylated in vivo. *Schröder* (1973) reported that N^4-acetyl sulphapyridine is deacetylated to the same extent in man, while *Funk et al.* (1970) found that N^4-acetyl sulphathiazole and N^4-acetyl chlorsulphisomidine were deacetylated only to a small extent in rats. Contrary to these observations *Gelber et al.* (1971) reported that N^4-acetyl sulphadimidine was not deacetylated in man.

The binding of N^4-acetyl sulphanilamide (28 %) and N^4-acetyl sulphadimidine (87 %) to the plasma proteins is higher than that of sulphanilamide (20 %) and sulphadimidine (66 %) (*Jørgensen & Rasmussen* 1972). The determination of the protein-binding was carried out by the same technique in both cases. From experiments with sulphanilamide, sulphathiazole, and sulphisomezole in dogs *Arita et al.* (1972) also found a higher protein-binding of the N^4-acetyl derivatives than of the parent compounds. Similar results are obtained with several sulphon-

amides and their N^4-acetyl derivatives added to human plasma (*Rieder* 1963). *Salvi & Plancher* (1963) and *Walker* (1970), however, reported that N^4-acetyl sulphamethoxypyridazien and N^4-acetyl sulphadimethoxine in man were bound to the plasma proteins to the same extent as the parent compounds.

As it is only the non-protein-bound fraction of the drugs which can filtrate through the glomeruli of the kidney, the renal excretion has been estimated on the basis of clearance of the non-protein-bound sulphonamide, and this clearance has been compared with the clearance of creatinine.

The experiments showed that the clearance of creatinine was 23—29 ml/min./10 kg b.wt., a result which is in accordance with previously reported values for goats (*Jørgensen & Rasmussen*). The clearance of non-protein-bound N^4-acetyl sulphanilamide (39 ± 2 ml/min./10 kg b.wt.) is higher than the clearance of creatinine, and this indicates that active tubular secretion is involved in the renal excretion of this compound. This observation is further confirmed in the experiments where diodone and N^4-acetyl sulphanilamide were infused simultaneously, resulting in a complete blockade of the active tubular secretion of N^4-acetyl sulphanilamide (Table 2). These results show that N^4-acetyl sulphanilamide is excreted by filtration and active tubular secretion, while filtration and reabsorption are involved in the renal handling of the parent compound (clearance: 12 ± 1 ml/min./10 kg b.wt.) (*Jørgensen & Rasmussen*).

The ratio between clearance of non-protein-bound N^4-acetyl sulphadimidine and clearance of creatinine varies between 9 and 15. Although the ratio is considerably reduced during loading with diodone it is still higher than 1, which means that the active tubular secretion of N^4-acetyl sulphadimidine was not entirely blocked by the diodone infusion. The reason might be that the concentration of diodone in plasma has not been high enough to give a complete blockade of the active tubular secretion of N^4-acetyl sulphadimidine although the same dosage entirely inhibited the active tubular secretion of N^4-acetyl sulphanilamide. The experiments indicate that N^4-acetyl sulphadimidine is excreted by filtration and active tubular secretion while reabsorption was not shown to take place. The renal excretion of N^4-acetyl sulphadimidine (clearance: 352 ± 46 ml/min./10 kg b.wt.) is much faster than that of the parent compound (clearance: 33 ± 2 ml/min./10 kg b.wt.) (*Jørgensen & Rasmussen*).

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SAMMENDRAG

Renal ekskretion af N^4-acetylsulfanilamid og N^4-acetylsulfadimidin hos geder.

Den renale udskillelse af N^4-acetylsulfanilamid og N^4-acetylsulfadimidin er undersøgt ved 19 forsøg på 6 geder. Begge derivaterne deacetyleres in vivo omend kun i mindre omfang. Undersøgelserne viste endvidere, at begge N^4-acetylderivaterne bindes til plasmaproteinerne i større udstrækning end de tilsvarende ikke-acetylerede forbindelser. Udskillelsen af de N^4-acetylerede sulfonamider sammenlignes med udskillelsen af kreatinin, og det vises, at den ikke-proteinbundne del udskilles ved filtration og aktiv tubular sekretion. Den renale udskillelse af N^4-acetylderivaterne er større end udskillelsen af de tilsvarende ikke-acetylerede forbindelser.

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