Oxidation and Acetylation of Sulfamonomethoxine by the Snail *Cepaea hortensis*

Tom B. VREE^{1),2)}, Monika L. VREE³⁾, Eleonora W. J. BENEKEN KOLMER¹⁾, Yechiel A. HEKSTER¹⁾, Minoru SHIMODA^{1,4)}, Jacques F. M. NOUWS⁵⁾, Toshiyuki YOSHIOKA⁶⁾, and Kazuhiko HOJI⁶⁾

Department of ¹⁾Clinical Pharmacy, ²⁾Anaesthesiology, Sint Radboud Hospital, Geert Grooteplein Zuid 8, Nijmegen, ³⁾Dotulabs, Weezenhof 35-08, Nijmegen, ⁵⁾R. V. V.-kring 6, Wolfskuilseweg 279, Nijmegen, the Netherlands, ⁴⁾Department of Agriculture, Tokyo University of Agriculture and Technology, Tokyo, and ⁶⁾Daiichi Seiyaku Co. Ltd., Tokyo, Japan

(Received 26 October 1988/Accepted 29 November 1988)

ABSTRACT. Sulfamonomethoxine is not O-demethylated in the snail *Cepaea hortensis*, but acetylated (15.2%) and oxidised (0.78%) at the 2 position of the pyrimidine nucleus.—**KEY WORDS:** *Cepaea hortensis*, N₄-acetylsulfamonomethoxine, sulfamonomethoxine, 2-hydroxysulfamonomethoxine.

Jpn. J. Vet. Sci. 51(2): 369–372, 1989

Sulfamonomethoxine (4-sulfanilamido-6-methoxypyrimidine) is slowly eliminated in man (T_{1/2} 30h) [6, 8], but relatively fast in cattle, horse and pig [1, 2, 3]. In contrast to sulfadimethoxine, the rate of acetylation of sulfamonomethoxine is relatively high in man [8] and presumably also in cattle, horse and pig [2, 3]. In man the mass balance of sulfamonomethoxine accounts for 70%, leaving ample room for an O-dealkylation pathway. Recently we have shown that the turtle *Pseudemys scripta elegans* is able to perform O-dealkylation reactions in sulfadimethoxine and sulfamonomethoxine [11, 12]. In sulfamonomethoxine an O-dealkylation reaction can be carried out at the 6 position only (Fig. 1). Therefore metabolism of sulfamonomethoxine may be assumed to be much simpler than that of sulfadimethoxine.

Snails are able to oxidise and acetylate sulfonamides [7, 9, 10], whereas their ability for an O-dealkylation reaction has been reported recently [15]. This investigation describes that in *Cepaea hortensis* sulfamonomethoxine is acetylated and oxidised at the 2 position of the pyrimidine nucleus, but

that there is no O-dealkylation.

MATERIALS AND METHODS

A tank was filled with sixty snails *Cepaea hortensis* (140 g total body weight). To the tank 45 mg sulfamonomethoxine, dissolved in 100 ml water and 2 ml 0.1 N NaOH, was added. The snails moved normally within the tank, propelling themselves up the walls of the tank, swallowing the water containing sulfonamide and then regurgitating it. At regular time intervals the snails were pushed back into the water, after which they fled it again, etc. The sulfonamide is excreted both by the kidney and in the faeces and redissolved in the water. The glass walls were rinsed with the solution in order to collect all the mucus, faeces and urine. After each rinsing procedure, an aliquot of 2 ml was taken and stored at –20°C until analysis (n=21). For each sample removed, 2 ml of water was added in order to keep the water content of the tank as constant as possible. The tank was closed to prevent evaporation of water.

At T=110 h, the total fluid was discarded

Fig. 1. Metabolic scheme of sulfamonomethoxine and its metabolites.

(80 ml), and 100 ml fresh tap water was added to the tank. Two samples were collected. At $T=130$ h, 8 snails were still alive, 100 ml water was discarded and replaced by 100 ml fresh tap water. Sampling continued till $T=190$ h. At the end of the experiment 5 snails were still alive. The samples were kept at -20°C until analysis. **Analysis:** Sulfamonomethoxine and its metabolites were analyzed by high performance liquid chromatography. The column was Cp Spher C8 5 μM , 250 mm $\times$ 4.6 mm ID (Chrompack, Middelburg, The Netherlands) with a guard column 50 mm $\times$ 4.6 mm, 10 μM . The mobile phase was a mixture of 0.01M KH_2PO_4 buffer pH 3.2 (pH adjusted with 0.3 ml 25% orthophosphoric acid)+0.30 g tetramethylammoniumchloride and 70 ml acetonitrile. Flow rate was 1.2 ml/min. UV detection at 282 nm.

Capacity factors of the compounds are respectively: 2-OH-sulfamonomethoxine 1.28, sulfamonomethoxine 8.19, and N_4 -acetylsulfamonomethoxine 21.58.

Deconjugation: Deglucuronidation was carried out in 3 different ways: 1 ml sample was mixed with 0.5 ml of 0.2 M KH_2PO_4 and 0.1 ml betaglucuronidase (*E. Coli*, pH 6.8, 10.000 E/ml; *Helix Pomatia* type H2 10.000

E/ml, pH 5.0; type B1, 10.000 E/ml pH 5.0; Sigma, St. Louis, USA). The mixtures were allowed to react for 6 hr at 37°C .

Desulfation: 1 ml sample was mixed with 0.5 ml of 0.2 M sodium acetate (pH 5.0) and 0.1 ml of arylsulfatase (pH 5.0; 200 E/ml; Sigma, St. Louis, USA). The efficiency of both conjugation reactions was tested with a sample of human urine containing sulfadimethoxine and its glucuronidated hydroxy metabolite.

Drugs: Sulfamonomethoxine was obtained by Prof. Ei-ichi Kokue from Daiichi Seiyaku, (Tokyo, Japan) and N_4 -acetylsulfamonomethoxine was synthesised [6].

2-Hydroxysulfamonomethoxine (2-demethylsulfadimethoxine) was synthesised by Daiichi Seiyaku, Tokyo, Japan.

RESULTS

Fig. 2 shows that sulfamonomethoxine disappears rapidly from the tank and that N_4 -acetylsulfamonomethoxine is the main metabolite. No 6-hydroxy- (6-demethyl-) sulfamonomethoxine could be detected, instead the metabolite 2-hydroxysulfamonomethoxine was present. No glucuro-

Fig. 2. Concentration-time profile of sulfamonomethoxine (S) and its metabolites 2-hydroxysulfamonomethoxine (2-OH) and N_4 -acetylsulfamonomethoxine (N_4), by the snails *Cepaea hortensis* after administration of an oral dose of 45 mg sulfamonomethoxine.

nide conjugates of sulfamonomethoxine and its metabolites were detected.

At the end of the first sampling period of 110 h, 91.5% of the administered dose has been disappeared from the tank, and only 8% N -acetyl- and 0.42% 2-OH-sulfamonomethoxine could be recovered. After the first water change only 3.2% sulfamonomethoxine and 4.8% N_4 -acetyl- and 0.21% 2-OH-sulfamonomethoxine could be recovered. After the second water change 1.1% sulfamonomethoxine, 2.4% N_4 -acetyl- and 0.15% 2-OH-sulfamonomethoxine is recovered from the tank.

DISCUSSION

Sulfamonomethoxine and sulfadimethoxine form the same metabolite 2-OH-sulfamono (di) methoxine, by respectively 2-oxidation and by 2-O-dealkylation in

snails [15] and turtles [12, 13]. This principal metabolite of these two metabolites must have a preferred structure as is shown in Fig. 3. This 2-position is accessible for oxidation and for O-dealkylation in sulfadi-(mono) methoxine. In both compounds the 2-position is twice as fast or reactive as the 6-position [11, 15]. In the snails the 6-O-dealkylation is too slow to produce recognisable concentrations, or it does not exist. As soon as a methyl group is substituted at this 2-position, as in sulfisomidine and sulfamethomidine, no oxidation takes place, neither at the 2-position, nor at the substituted methyl groups [13, 14]. N_4 -acetylation of sulfamonomethoxine is the main metabolic pathway in snails, while this reaction for sulfamonomethoxine was absent in turtles [12]. In earlier literature N_1 -glucosiduronic and glucopyranosiduronic acid conjugates were described as metabolites of sulfamonomethoxine in man [4, 5], but these conjugates were presumably formed with the hydroxy metabolites. In man, sulfamonomethoxine is acetylated for 60–80%, leaving room for 20% 6-O-dealkylation, after which these hydroxy metabolites can be conjugated with glucuronic acid [8].

In total, 15.2% of the dose is present in the tank as N_4 -acetyl-sulfamonomethoxine, 0.78% as 2-hydroxysulfamonomethoxine, and 13.8% as parent drug (sum 29.78%). This means that still 70% of the dose is unaccounted for; similar figures are reported for the other sulfonamides in snails [7, 9, 10]. The 13 samples of 2 ml taken from the tank in the first sampling period remove approximately 23.1% of the dose, leaving approximately 50% unaccounted for. The remaining loss in the mass balance can be partly explained by the dying of 52 snails in the first sampling period, trapping the sulfonamide in their bodies. In the second and third sampling period the living snails are able to excrete the parent drug and the two metabolites. The large number of snails in

the small tank prevent the underlying snails to escape from the water, which resulted in the drawing of these pulmonates.

REFERENCES

1. Rauws, A. G., van Schothorst, M., and Frik, J. F. 1975. Plasma concentrations, plasma protein binding and residues of sulfamonomethoxine in pigs, horses and cattle. *Tijdschrift Diergeneeskunde* 100: 1099-1104.
2. Shimoda, M., Tsuboi, T., Kokue, E., and Hayama, T. 1983. Dose dependent pharmacokinetics of intravenous sulfamonomethoxine in pigs. *Jpn. J. Pharmacol.* 33: 903-905.
3. Shimoda, M., Shimizu, T., Kokue, E., and Hayama, T. 1984. Possibility of saturation in renal excretion after high dose of intravenous sulfamonomethoxine in pigs. *Jpn J. Vet. Sci.* 46: 331-337.
4. Ueda, M., Murakami, N., Atsumura, H., and Huruki, K. 1967. Studies on the metabolism of drugs. VII. On the metabolite of sulfamonomethoxine in human. *Yakugaku Zasshi* 87: 455-457.
5. Ueda, M., Tsurui, Y., and Joizumu, T. 1972. Studies on metabolism of drugs. XII. Quantitative separation of metabolites in human and rabbit urine after oral administration of sulfamonomethoxine and sulfadimethoxine. *Chem. Pharmacol. Bul.* (Tokyo) 20: 2042-2046.
6. Vree, T. B., Hekster, Y. A., and Tjhuis, M. W. 1985. Metabolism of sulfonamides. *Antibiot. Chemother.* 34: 66-120.
7. Vree, T. B., Vree, M. L., and Nouws, J. F. M. 1986. Acetylation and hydroxylation of sulphadimidine in the snail *Cepaea hortensis*. *J. Vet. Med. A.* 33: 633-636.
8. Vree, T. B. and Hekster, Y. A. 1987. Clinical Pharmacokinetics of sulfonamides and their metabolites. *Antibiot. Chemother.* 37: 25-30.
9. Vree, T. B., Vree, M. L., and Nouws, J. F. M. 1987. Acetylation and hydroxylation of sulphathiazole in the snail *Cepaea hortensis* and the slug *Arion rufus*. *Vet. Quart.* 9: 88-90.
10. Vree, T. B., Vree, J. B., Hekster, Y. A., and Nouws, J. F. M. 1987. Acetylation, deacetylation and hydroxylation of sulphamethoxazole and N₄-acetylsulphamethoxazole in the snail *Cepaea hortensis*. *Vet. Quart.* 9: 378-381.
11. Vree, T. B., Vree, J. B., Beneken Kolmer, N., Hekster, Y. A., Shimoda, M., Nouws, J. F. M., Yoshioka, T., and Hoji, K. 1988. O-demethylation and N₄-acetylation of sulfadimethoxine by the turtle *Pseudemys scripta elegans*. *Vet. Quart.* in press.
12. Vree, T. B., Vree, J. B., Beneken Kolmer, N., Hekster, Y. A., Shimoda, M., Nouws, J. F. M., Yoshioka, Y., and Hoji, K. 1988. Oxidation and O-dealkylation of sulfamonomethoxine by the turtle *Pseudemys scripta elegans*. *Vet. Quart.* in press.
13. Vree, T. B., Vree, J. B., de Jonge, P. A. M., Plaum, M. J. M., Verwey, C. P. W. G. M., Hekster, Y. A., Shimoda, M., and Nouws J. F. M. 1988. Lack of oxidative pathways in the metabolism of sulfisomidine by the turtle *Pseudemys scripta elegans*. *J. Vet. Pharmacol. Ther.* submitted for publication.
14. Vree, T. B., Vree, M. L., Beneken Kolmer, N., Ross, M. E., Hekster, Y. A., Shimoda, M., Nouws, J. F. M. 1988. O-dealkylation and acetylation of sulfamethomidine by the snail *Cepaea hortensis*. *J. Vet. Pharmacol. Ther.* submitted for publication.
15. Vree, T. B., Vree, M. L., Beneken Kolmer, N., Hekster, Y. A., Shimoda, M., Nouws J. F. M., Yoshioka, T., and Hoji, K. 1989. O-demethylation and N₄-acetylation of sulfadimethoxine by the snail *Cepaea hortensis*. *Jpn. J. Vet. Sci.* 51: 364-368.

要 約

カタツムリ *Cepaea hortensis* によるスルファモノメトキシンの酸化とアセチル化: Tom B. Vree¹⁾²⁾・Monika L. Vree³⁾・Eleonora W. J. Beneken Kolmer¹⁾・Yechiel A. Hekster¹⁾・下田 実⁴⁾・Jacques F.M. Nouws⁵⁾・吉岡利幸⁶⁾・傍士和彦⁶⁾ (Department of ¹⁾Clinical Pharmacy, ²⁾Anaesthesiology, Sint Radboud Hospital, ³⁾Dotu-labs, ⁴⁾東京農工大学農学部, ⁵⁾R.V.V.-Kring 6, ⁶⁾第一製薬株式会社)——カタツムリ *Cepaea hortensis* において、スルファモノメトキシンは脱メチル化されなかった。しかしながら、アセチル化 (15%) およびピリミジン核の 2 位の酸化 (0.78%) が認められた。