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

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**ABSTRACT.** Sulfamonemethoxine 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, N$_4$-acetylsulfamonemethoxine, sulfamonemethoxine, 2-hydroxysulfamonemethoxine.**


Sulfamonemethoxine (4-sulfanilamido-6-methoxypyrimidine) is slowly eliminated in man (T1/2 30h) [6, 8], but relatively fast in cattle, horse and pig [1, 2, 3]. In contrast to sulfadimethoxine, the rate of acetylation of sulfamonemethoxine is relatively high in man [8] and presumably also in cattle, horse and pig [2, 3]. In man the mass balance of sulfamonemethoxine 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 sulfamonemethoxine [11, 12]. In sulfamonemethoxine an O-dealkylation reaction can be carried out at the 6 position only (Fig. 1). Therefore metabolism of sulfamonemethoxine 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* sulfamonemethoxine 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 sulfamonemethoxine, 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 sulfamonemethoxime 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 \(-20^\circ\)C until analysis. Analysis: Sulfamonemethoxime and its metabolites were analyzed by high performance liquid chromatography. The column was Cp Spher C8 5 \( \mu \)M, 250 mm \( \times \) 4.6 mm ID (Chrompack, Middelburg, The Netherlands) with a guard column 50 mm \( \times \) 4.6 mm, 10 \( \mu \)M. The mobile phase was a mixture of 0.01M \( \text{KH}_2\text{PO}_4 \) 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-sulfamonemethoxime 1.28, sulfamonemethoxime 8.19, and \( \text{N}_4\)-acetylsulfamonemethoxime 21.58. Deconjugation: Deglucuridanidation was carried out in 3 different ways: 1 ml sample was mixed with 0.5 ml of 0.2 M \( \text{KH}_2\text{PO}_4 \) and 0.1 ml betaglucuridonidase (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\(^\circ\)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 sulfadrimethoxime and its glucuronidated hydroxy metabolite.

Drugs: Sulfamonemethoxime was obtained by Prof. Ei-ichi Kokue from Daiichi Seiyaku, (Tokyo, Japan) and \( \text{N}_4\)-acetylsulfamonemethoxime was synthetised [6].

2-Hydroxysulfamonemethoxime (2-demethylsulfadimethoxime) was synthetised by Daiichi Seiyaku, Tokyo, Japan.

RESULTS

Fig. 2 shows that sulfamonemethoxime disappears rapidly from the tank and that \( \text{N}_4\)-acetylsulfamonemethoxime is the main metabolite. No 6-hydroxy- (6-demethyl-) sulfamonemethoxime could be detected, instead the metabolite 2-hydroxysulfamonemethoxime was present. No glucuro-
SULFAMONOMETHOXINE IN SNAILS

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 sulfadimethoxine. 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 sulphasomidine and sulphamethoxine, 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


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. Vree(1,2)・Monika L. Vree(3)・Eleonora W. J. Beneken Kolmer(3)・Yechiel A. Hekster(3)・下田 実(4)・Jacques F.M. Nouws(5)・吉岡利幸(6)・傍島和彦(6)(Department of 1)Clinical Pharmacy, 2)Anaesthesiology, Sint Radboud Hospital, 3)Dottola, 4)東京農工大学農学部, 5)R.V.V-Kring 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%) およびビリミジン核の2位の酸化 (0.78%) が認められた。