

## THE CONTRIBUTION OF GUT MICROFLORA TO PARACETAMOL METABOLISM

GORDANA BOJIĆ<sup>1</sup>, VESNA MILOŠEVIĆ<sup>1</sup>, VERA GUSMAN<sup>1</sup>, ANIKA POVAŽAN<sup>2</sup> and  
MAJA STOJANČEVIĆ<sup>3</sup>

<sup>1</sup>*Institute of Public Health, 21000 Novi Sad, Serbia*

<sup>2</sup>*Institute of Lung Disease, 21204 Sremska Kamenica, Serbia*

<sup>3</sup>*Department of Pharmacology and Toxicology, Faculty of Medicine, 21000 Novi Sad, Serbia*

*Abstract* - During the last decade, the important role of gut microflora as a special organ of the gastrointestinal system in the metabolism of drugs is well known. The aim of this study was to evaluate the role of the gut microecological population with enzymatic systems, especially beta-lyase, in the metabolism of paracetamol in mice. Two groups of 20 white male laboratory mice BALB/c, body weight 32±1.5 kg, were treated orally with neomycin sulphate (500 mg/kg in saline solution) and saline solution (10 ml/kg) twice daily for three days. After the treatment, the animals were given paracetamol dissolved in saline solution (200 mg/kg) intraperitoneally. The total amount of excreted paracetamol in 8 hours' collected urine was unchanged. A difference between treated and control mice was observed regarding a highly significant reduction in the excretion of 3-methylthiomethyl metabolites. A decrease in the excretion of thiomethyl metabolites was found in the control group compared to the experimental mice. Gut microflora had a great influence on the formation of metabolic precursors, thiomethyl-conjugates, and their oxidizable products. It is obvious that the ecosystem of gut microflora has an important role in the metabolism of paracetamol resulting in a significant reduction in the excretion of 3-methylthioparacetamol by urine, the glucuronide and sulphate.

*Key words:* Gut microflora, metabolism, paracetamol

### INTRODUCTION

Many recent studies are dedicated to the metabolic role of gut microflora. Conditions in the intestinal lumen allow for the presence of many thousands of bacterial strains, both aerobic and anaerobic (Scheline, 1973). Anaerobic bacteria are well known as bacteria that can live under extreme conditions regarding different temperatures, salinity, pH, the presence of toxic substrates or available free energy (Larsen, 1985). Enzymes such as beta-lyase that are the result of gut flora activity have the possibility to transform conjugates of cysteine from xenobiotics in toxic metabolites. These toxic products are thiols or

their metabolic products (Smith et al., 1978).

Gut microflora can destroy conjugates of glutathione in appropriate S-substituents of cysteine (Rowland, 1988). Data about the generation of thiols from conjugates of cysteine have been known for half a century, but recently the importance of metabolic pathways for forming different thiomethyl conjugates from many xenobiotics has been determined (Illet et al., 1990). In clinical practice, one of the most used drugs is paracetamol, whose metabolism depends on cytochrome-450, and which is inactivated by conjugation with glutathione (Mikov et al., 1988). This type of conjugate is excreted by urine as cysteine,

**Table 1.** The excretion of paracetamol metabolites in 8 hours' urine represented as relative value (%) of total intraperitoneally administered paracetamol (N=20, \*p<0.001).

Metabolites	Control group (X+/-SD)	Neomycin treated Group (X+/- SD)
Paracetamol		
-glucuronide	45.6+/-9.25	43.5+/-4.25
-sulphate	8.15+/-2.31	8.7+/-6.55
-cysteinate	17.56+/-4.32	14.51+/-3.22
-unchangeable	4.56+/-1.11	4.36+/-1.24
-mercapturate	0.31+/-0.45	0.102+/-0.086
-thiomethyl sulfate and methyl sulfoxide	2.11+/-1.11	0.21+/-0.15*
-thiomethyl glucuronide	0.54+/-0.24	0.023+/-0.067*
-thiomethyl	0.1+/-0.12	0.034+/-0.054
total thiomethyl paracetamol	2.56+/-1.46	0.344+/-0.23*

mercapturate and as methylthio-derivates. Gut microflora influence the paracetamol traveling through the gut system.

The aim of this research was to establish the place and the role of gut microflora in the formation of methylthio metabolites of paracetamol.

#### MATERIALS AND METHODS

In this study, two groups of 20 each male white laboratory mice BALB/c, body weight 32+/-1.5gr, were treated with neomycin sulphate (500 mg/kg in saline solution) and saline solution (10 ml/kg) per os, twice daily for three days. The following day all the animals were given paracetamol (200 mg/kg) dissolved in saline solution intraperitoneally. After inoculation of paracetamol, each mouse was kept separately in a metabolic cage, which is suitable for collection of urine and feces. Samples of 8 hours' urine were collected in dark tubes and kept at 0°C. After 8 h incubation, each cage was washed with 5 ml of water (Drasar, 1967). This liquid was added to the urine and kept at 20°C until the beginning of analysis (Aranki et al., 1965). The animals had free access to food and water throughout the experiment. Separated metabolites of paracetamol were determined by HPLC. The results are presented as the mean+/-SD. Student's t-test was

used to determine the significant differences between the groups.

#### RESULTS

In the urine collected over 8 h, the concentrations of metabolites of paracetamol were measured (Gibson, 1985). It was found that the main metabolites excreted by urine were paracetamol glucuronide, paracetamol cysteinate and paracetamol sulfate, unchanged paracetamol, methylthio paracetamol and its glucuronide, sulfate and sulfoxide (Table 1). After treatment with neomycin, the total quantity of excreted paracetamol in 8 hours' urine was not changed. Comparison of neomycin-pretreated mice and the control group revealed a highly significant reduction in the excretion of different 3-methylthio-metabolites in the neomycin-treated group (Stevens et al., 1986).

#### DISCUSSION

Paracetamol could be metabolized through three different pathways: conjugation with glucuronic acid, conjugation with sulfates, and metabolic activation by microsomal monooxygenases. This is the pathway for formation of reactive intermediary products that could be destroyed by conjugation with glutathione.

Such a conjugate with glutathione is mostly transformed before elimination. It is obvious that gut microflora contribute to the described occurrences in the metabolism of paracetamol as is seen in the confirmed differences between the experimental and control groups of mice. It is clear in the formation of 3-(methylthio)paracetamol. S-glutathionates are metabolized by hydrolysis in S-cysteinate which could be converted via three pathways: N-acetylation (when mercapturic acid is generated), transamination C-S cleavage, and separation of C-S cleavage.

It is well known that 3-thyoparacetamol is a very reactive compound that can contribute to drug toxicity. It is very difficult to localize C-S lyases of conjugates of cysteine in tissue. Gut microflora has a crucial contribution in this reaction (Freter, 1992).

In the group of neomycin-pretreated mice, the excretion of thiomethyl metabolites was decreased. While we cannot excluded the role of neomycin on the reabsorption of glutathione metabolites of paracetamol that are excreted by bile in intestine, a role for gut microflora in the metabolism of paracetamol has been shown (Mikov, 1991). Gut microflora generates the metabolic precursors of thiomethyl conjugates and their oxidized products (Schrezenmeiz, 2012).

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