INFLUENCE OF DIMETHOATE ON PYRANTEL CONCENTRATION IN RAT LIVER

ARKADIUSZ ZASADOWSKI, DARIUSZ BARSKI AND WOJCIECH PIEKOSZEWSKI

Division of Veterinary and Environmental Toxicology, Department of Pathology and Pharmacology, Faculty of Veterinary Medicine, University of Warmia and Mazury, 10-957 Olsztyn, Poland, 

Institute of Forensic Research, 31-033 Krakow, Poland

e-mail: toxvet@uwm.edu.pl

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Abstract

The aim of the study was to evaluate the influence of dimethoate on pyrantel concentrations in the liver after co-administration of dimethoate and pyrantel embonate. Rats were given intragastrically or in drinking water pyrantel or pyrantel and dimethoate, on different regimes of dosing. In the first group, after administration of pyrantel embonate at a dose of 1 000 mg/kg, the maximum concentration of pyrantel in the liver was 440 ng/g and the half-life in the terminal phase was 151 h. After simultaneous administration of pyrantel and dimethoate, the maximum concentration of pyrantel decreased to 174 ng/g; however, the half-life did not change. Administration of a 2.5 times lower dose caused a non-proportional lowered maximum concentration of pyrantel and a statistically significant increase in its rate of elimination. The influence of dimethoate on pyrantel concentration in rat liver was similar after pyrantel administration both at a dose of 400 mg/kg and 1 000 mg/kg. The obtained results show that dimethoate probably does not influence pyrantel biotransformation; however, they can suggest the inhibition of absorption of this compound and changes in its body distribution.

Key words: rats, liver, pyrantel embonate, dimethoate.

Chronic and acute poisoning due to an exposure to environmental xenobiotics is rarely the result of a single chemical; it is most frequently caused by many compounds, which can interact with each other. Pyrantel embonate (1,4,5,6-tetrahydro-1-methyl-2-[2-(2-thienyl)ethenyl] pyrimidine) is an effective drug in the treatment of intestinal helminthiasis (2, 7). LD50 per os for rats is 2 000 mg/kg b.w. Ten fourteen per cent of pyrantel is absorbed in the gastrointestinal tract. Within the body it is metabolized mostly in the liver via three routes (oxidation of thiophen ring, tetrapyrimidine ring and conjugation with mercapturic acid) (4). Maximum concentration of pyrantel embonate in plasma after administration per os is ascertained between 4 and 8 h after exposure and depends on species as well as the doses (3). Pyrantel can interact with other xenobiotics, especially with medicaments and organophosphorus pesticides.

Dimethoate (O,O-dimethyl S-N-methyl carbamoyl methyl phosphodithioate) is an organophosphorus insecticide with a wide range of activities, mainly used to protect plants against pests (6, 12); it is also used as an indoor insecticide (10). The DL50 per os for rats is 387 mg/kg b.w. The lungs, gastrointestinal tract and skin are the routes of dimethoate transport into the organism and next it is accumulated mainly in the liver and kidneys. Monoxygenases with mixed function and cytochrome P450 system are major enzymes taking part in dimethoate biotransformation (9).

In the scientific literature, information about the influence of pyrantel on physiological processes in mammals and possible interaction with other drugs and environmental xenobiotics is fragmentary (1, 8).

Taking into consideration the widespread use of pyrantel in the treatment of gastrointestinal helminthiasis and the frequency of the use of dimethoate as an insecticide, it seems reasonable to study the influence of dimethoate on pyrantel concentrations in the liver, bearing in mind that the liver is the most important organ of pyrantel metabolism.

Material and Methods

Dimethoate (C5H12NO3PS2) (CHEMINOVA, Denmark) containing 99.1% of pure O,O-dimethyl S-N-methyl carbamoyl methyl phosphodithioate and pyrantel embonate (C11H14N2S.C23H1606) (POLPHARMA, Poland), containing 99.3% of pyrantel embonate were used in the experiment.

Studies were conducted on 216 male Wistar rats (initial body weight 180±10 g) originating from the Animal Breeding Center in Brwinów near Warsaw. The rats during the acclimatization and experiment were housed under the laboratory standard conditions (12 h light/dark cycle, 22 ± 1°C, humidity 70 ± 10% and gravitational-mechanical ventilation), with free access to
standard chow “Murigran”. The experiment was carried out in two stages. At each stage the rats were divided into a control group (C) and two experimental groups: stage 1 – groups I and II; stage 2 – groups III and IV (36 animals in each group). Animals from group I received water suspension of pyrantel embonate given by gavage at a dose of 1000 mg/kg b.w. (½LD₅₀) on the 14th and 28th d of the experiment; animals from group II were given dimethoate at a dose of 15.48 mg/kg b.w. (1/25 LD₅₀) in drinking water for 28 d, and pyrantel embonate at the same dose and way as the group I. Animals from group III received pyrantel embonate at a dose of 400 mg/kg b.w. (1/5 LD₅₀) for 3 consecutive days and rats from group IV - dimethoate at a dose of 38.7 mg/kg b.w. (1/10 LD₅₀) for 5 consecutive days, and pyrantel embonate at a dose of 400 mg/kg b.w. on days 3, 4 and 5 from the beginning of dimethoate intoxication. In the second stage of the experiment dimethoate as well as pyrantel embonate were given to animals by the gavage. Rats from control groups received only standard chow and water ad libitum.

The experiments followed provisions of the “Act on Animal Protection” and recommendation of the Local Ethical Committee for Animal Experiments of the University of Warmia and Mazury in Olsztyn.

Rats were sacrificed by decapitation at 3, 6, and 12 h and 2, 7 and 14 d after intoxication, and their livers were sampled for pyrantel residues determination. At each time point 6 animals were sacrificed. The livers were stored at -70°C until analyses. The liver was homogenised with distilled water and 30 ml of chloroform and 100 mg of sodium carbonate were added to the homogenate (~2 g of liver), and the samples were shaken for 0.5 h and after this centrifuged for 15 min at 4000 rpm. The organic phase was separated and evaporated to dryness in a nitrogen stream at 50°C and dissolved in 100 µl of dimethylsulphoxide. The determination of pyrantel was performed by the self-developed HPLC method (Crystal 2000 ATI Unicam) with spectrophotometric detection. A mixture of water and methanol (1:2) was used as the mobile phase. Standard samples were prepared by spiking a blank liver with a standard solution of pyrantel. The linearity range of the method was 10-400 ng/ml. The limit of detection (LOD) was 2.5 ng/ml, and the lower concentration on the calibration curve was assumed as the limit of quantification (LOQ). Inter-day precision was 11.2 ± 4.7% and intra-day 10.4 ± 5.4%. Pharmacokinetic calculations were performed on the basis of statistical moment analysis (model independent pharmacokinetics) with application of SPLINE computer programme.

Data were analysed statistically by a one-way analysis of variance ANOVA followed by the Newman-Keuls t test. All the data were expressed as mean ± SEM. Differences with P≤0.05 and P≤0.01 were regarded as statistically significant.

**Results**

The results of pyrantel determination in the liver of rats in respective groups are shown in Figs 1 and 2.

**Fig. 1.** Pyrantel concentrations in rat liver exposed to pyrantel embonate (group I) and dimethoate and pyrantel embonate (group II), expressed in ng/g (mean ± SEM, n=6); * P≤0.05; ** P≤0.01.
In rats that received pyrantel embonate intragastrically twice at a dose of 1 000 mg/kg (group I), the concentration of this compound was detected up till the 14th d after the last applied dose. The maximum concentration in this group was observed at the 3rd h and was 440.87 ng/g. A rapid decline in pyrantel concentration in the liver was observed between the 3rd and 12th h (Fig. 1). At the 12th h its concentration was 9 times lower than that at the 3rd h. On the 2nd d the mean concentration was 19.53 ng/g of the liver and was very slowly decreasing. On the last day of the experiment (day 14), concentration of pyrantel was 4.8 ng/g.

As a result of simultaneous administration of pyrantel embonate and dimethoate (group II) at the 3rd and 6th h after intoxication, the concentration of pyrantel in the liver was 174.0 ng/g and 150.33 ng/g, respectively, and was significantly lower than that after individual pyrantel administration (group I). Between day 2 and 14 similar concentration of pyrantel was ascertained in both groups of rats (groups I and II) and any differences observed were statistically insignificant (Fig. 1).

At the second stage of the experiment, after individual administration of pyrantel embonate at a dose of 400 mg/kg for 3 consecutive days (group III), as well as co-administration with dimethoate (group IV) the concentrations of this compound was detected up till the 7th d after exposure. The maximum concentration of pyrantel in the liver of rats of group III occurred at the 3rd h of the experiment (108.1 ng/g) and was over 50% greater than that in group IV and the difference was statistically significant (Fig. 2). At later time points (12 h, 2 and 7 d), the level of pyrantel in groups III and IV was similar, although a little higher concentration was observed up till the 2nd d. The pyrantel concentration in the liver after 7 d was 4.4 ng/g in group III and 5.67 ng/g in group IV (Fig. 2).

Discussion

In the literature only a few studies refer to the pyrantel in animal tissues, and show, that maximum concentration of the compound is attained at the first hours after exposure and then relatively quickly decomposed in animal organisms.

Our studies showed that pyrantel embonate given twice at a dose of 1 000 mg/kg in 2-week interval as early as at the 3rd h after the last applied dose attained the maximum concentration in the liver of rats. A similar maximum concentration was observed in the blood of dogs after administration of pyrantel pamoate at a dose of 10 mg/kg (11). The dose of pyrantel embonate used in our studies was much higher, which suggests a much lower bioavailability of this compound in comparison with the pamoate salt of pyrantel. The half life in this period (distribution) was around 3 h. So, a rapid decrease in pyrantel concentration can be caused by intensive transport from the blood and well blood supplied organs (liver) to tissues rich in lipids. The elimination of pyrantel in the terminal phase was very slow, with a biological half-life in the liver equal to 151.6 h. This slow elimination may have been caused by slow biotransformation; however, other studies, in which the rate of elimination was calculated on the basis of pyrantel concentration in blood showed rapid elimination (3, 11). Another reason for a slow rate of
elimination may be permanent liberation of pyrantel from the tissue deposit.

The level of pyrantel concentrations after co-administration of pyrantel embonate and dimethoate was significantly lower than after individual pyrantel administration. The observed differences are probably not caused by a decrease in bioavailability, which is at all very low, but by changes in the body distribution; however, the performed experiment was not able to prove it. According to other authors, it appears that in addition the liver and kidneys also plays an important role in pyrantel distribution (5). In rats given both compounds the equalization of pyrantel concentration between the liver and other organs was slower than in rats received only pyrantel embonate, and the half-life (10.4 h) was 3 times longer; however, after 12 h the differences between pyrantel concentrations in both groups were not statistically significant. Also, at the next time points, the concentration of pyrantel in both groups was similar (Fig. 1). The elimination rate of pyrantel (t1/2 177 h) in the group treated with both compounds was only a little slower (16%) than that after administration of pyrantel only. This indicates that dimethoate did not influence pyrantel biotransformation or kidney excretion.

At the second stage of the experiment after administration of pyrantel embonate for 3 consecutive days at a dose two and a half times lower (400 mg/kg) than at the first stage, the maximum concentration of pyrantel decreased as much as 4 times. The decrease in the concentration was much greater than could be predicted from the change of the dose. It can be assumed that this phenomenon could be caused by a different pattern of pyrantel dosing (at the first stage pyrantel was administered twice, on days 14 and 28, while at the second stage for 3 consecutive days from the beginning of dimethoate intoxication). The lowering of the maximum concentration caused a shortened time of pyrantel detection (7 d). After 14 d, the concentration of pyrantel in groups III and IV was below the limit of detection of the analytical method.

At both stages of the experiment the concentrations of pyrantel in rat liver at the first time points after intoxication (3 and 6 h) were significantly lower after simultaneous administration of pyrantel and dimethoate, than after administration of pyrantel alone. At later time points, the concentrations of pyrantel in all the studied groups were similar. The elimination rate of pyrantel in the terminal phase (after equilibration of concentrations) after the lower dose (400 mg/kg) was higher (t1/2 151 h – pyrantel alone; 177 h - pyrantel with dimethoate) than after dose of 1 000 mg/kg. The observed differences are very hard to explain on the basis of current study.

It can be concluded that bioavailability of pyrantel after administration as pyrantel embonate is very low and does not depend in a direct manner on the administered dose. After intragastric administration, the maximum concentration of pyrantel is reached relatively fast (first time point of the experiment) followed by a rapid distribution to the other tissues, which persists for 12 h. The biological half-life of pyrantel in this phase is equal to around 3 h independently of the dose. After this, elimination of pyrantel slows down and the half-life of pyrantel, depending on the studied group, ranges from 99 to 177 h. The administration of pyrantel together with dimethoate led to a significant decrease in the maximum concentration of pyrantel with a slight influence on the distribution and elimination phase. The obtained results did not prove the influence of dimethoate on pyrantel biotransformation, but suggest the inhibition of pyrantel absorption or changes in tissue distribution. Further study should clear up the mechanism of interaction between pyrantel embonate and dimethoate.

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References