EFFECT OF PYRANTEL AND DIMETHOATE ADMINISTRATION ON RAT LIVER CYTOCHROME P450 SYSTEM

RYSZARD WIADERKIEWICZ1, ARKADIUSZ ZASADOWSKI2, PIOTR CZEKAJ1, ANNA WIADERKIEWICZ1, BEATA CZAJKOWSKA1, DARIUSZ BARSKI2 AND ARTUR PAŁASZ1

1 Department of Histology and Embryology, Medical University of Silesia 40-752, Katowice, Poland
2 Department of Veterinary Toxicology and Environmental Protection, University of Warmia and Mazury 10-957, Olsztyn, Poland
e-mail: histem2@slam.katowice.pl

Received for publication January 09, 2005.

Abstract

The cytochrome P450 system in rat liver after administration of dimethoate (5 d, 1/10 DL50), pyrantel embonate (3 d, 1/5 DL50) or both xenobiotics simultaneously was analysed. Both compounds were administered directly to the stomach by the tube and the components of cytochrome P450 system were analysed in the microsomal fraction of the liver up to 14 d after the last applied dose. Intoxication with pyrantel diminished the total content of cytochrome P450 in all analysed time intervals. On the other hand, intoxication with dimethoate resulted in increase in the cytochrome P450 content 2 d after the last applied dose. The changes in activities of NADPH: cyt.P450 and NADH: cyt.b5 reductases were small and statistically not significant. Both dimethoate and pyrantel affected the expression of CYP1A2, CYP2B1/2 and CYP3A1 proteins. Both compounds had a slight negative effect on CYP2B1/2. In animals receiving dimethoate as well as both xenobiotics simultaneously a significant increase in the level of CYP1A2 protein was observed. However, stimulatory effect of dimethoate on the expression of CYP1A2 was abolished by simultaneous intoxication with pyrantel. The changes in CYP3A1 protein expression corresponded with those observed for the total amount of cytochrome P450.

Key words: rats, pyrantel, dimethoate, cytochrome P450, liver, drug interaction.

The same CYPs are often involved in biotransformation of such different compounds as pesticides and commonly used drugs. Dimethoate and pyrantel are typical examples of such compounds frequently used in the agriculture and veterinary medicine, respectively.

Environmental pollution with organophosphorus insecticides, which are broadly used in the agriculture to eradicate insects, create potential health hazards including acute and chronic cases of human and animal poisonings (13,14). Organophosphates containing phosphorus derived from phosphoric acid are generally the most toxic to vertebrate animals of all used pesticides. Dimethoate (O,O-dimethyl s-N-methyl carbamoyl methyl phosphodithioate) is metabolically activated by P450 mediated oxidative desulphurization (6). Dimetoxon, an oxygen analogue metabolite of dimethoate, appears to play a dominant role in its toxicity both for mammals and insects (17).

Pyrantel embonate (1,4,5,6-tetrahydro-1-methyl-2-[2-(2-thienyl)ethenyl (pyrimidine) is commonly used to treat helminth infections both in humans and animals. It is one of four drugs listed in the WHO Model List of Essential Drugs that are recommended for the control of helminthiasis (16). Pyrantel is a potent activator of the acetylcholine receptors on the muscle cells of nematodes, which induces spastic and prolonged paralysis of the worm and its elimination from the host (12). Unfortunately, pyrantel can also affect neurotransmission in vertebrates due to its ability to act as an open-channel blocker of mammalian acetylcholine receptors and their low efficacious agonist (11).

The high incidence of parasitic infections and widespread use of organophosphorus pesticides in environment creates the risk of undesirable and unexpected interactions between multiple xenobiotics. Although pyrantel embonate is metabolized in vivo mainly by CYP-2D6, it is suggested that it may affect

The liver is the main organ of the body where the biotransformation and detoxification of many endo- and exogenous compounds take place. The key enzymes of phase I reactions, which initiate the metabolism of xenobiotics, are cytochrome P450 dependent monooxygenases. These enzymes are located mostly in the microsomal fraction of the hepatocytes and are products of multigene superfamily coding for the proteins with limited and overlapping specificity to particular xenobiotics. In mammals, the cytochrome P450 isofoms (CYPs') involved in the metabolism of xenobiotics belong mostly to the families CYP1-CYP3.
expression of other CYP isoforms (2, 8). If so, under co-
exposure conditions pyrantel would interact with
pesticides metabolized by cytochrome P450 isoforms
with possible clinically significant after-effects.

Material and Methods

Male Wistar rats (2 month old, initial body
weight 180 ± 10 g) were used in the study. Animals
were kept in plastic cages in stabilized temperature 20-
22 °C, humidity 70%, light cycle (day/night) 12/12 h and
allowed free access to standard laboratory rat chow and
water ad libitum.

Animals were randomly divided into control
group (C), and three experimental groups (D, P and PD),
with 36 animals in each group. Group D was given
dimethoate (99.1%, Cheminova Inc., Denmark) at the
dose of 38.7 mg/kg b.w. (1/10 LD50) once daily for 5
consecutive days. Group P was given pyrantel embonate
(99.3%, Polpharma S/A, Poland) at the dose of 400
mg/kg b.w. (1/5 LD50) once daily for 3 consecutive days.
Both dimethoate and pyrantel embonate were
administered directly to the stomach by the tube as water
suspension. Animals in group (PD) received both
dimethoate and pyrantel embonate at the doses and
regime of administration as in the above groups but
pyrantel embonate was given during the last 3 d of
dimethoate intoxication. Control group received water
only. The animals were sacrificed 3, 6, 12 h and 2, 7, 14
d after the last applied dose, and the liver was excised
for further analysis. All the experiments on animals
were approved by the local ethics committee.

In the microsomal fraction isolated from the
liver homogenates according to Dalner (3) the following
values were determined: content of protein by Lowry
et.al. method (10); cytochrome P450 content and its
NADPH reductase activity by the methods of Estabrook
and Werringloer (4) and Hodges and Leonard (5)
respectively; cytochrome b5 content and its NADH
reductase activity by the method of Hodges and Leonard
(5). The protein expression of particular P450 isoforms
was measured by Western blot immunoassay. Microsomal samples (5 mg of protein) were subjected to
polyacrylamide gel (8%) electrophoresis in the presence
of sodium dodecyl sulphate as described by Laemmli
(7). The resolved proteins were blotted electrophoretically onto PVDF membrane (Milipore)
and stained immunochemically. Antibodies to CYP1A2,
CYP2B1/2 and CYP3A1 were developed in rabbits
and obtained from Chemicon Int.Inc. The binding of all
antibodies was detected with goat anti-rabbit secondary
antibodies conjugated with alkaline phosphatase and the
reaction was developed with BCIP/NBT liquid substrate
system (Sigma) according to manufacturer instruction.
The molecular weights and intensities of stained bands
were analysed with One D-scan software (Scanalytics).
Data analysis and evaluation of statistical significance
was performed with one-way ANOVA test using
Statistica 6.0 software.

Results

Cytochrome P450. The changes in total
content of cytochrome P450 in the microsomal fraction
of the liver are shown in Table 1. In rats intoxicated with
pyrantel the content of the cytochrome significantly
diminished (P<0.05) up to the 12th h after the last
applied dose. At the same time the effect of dimethoate
on the total content of cytochrome P450 was negligible.
However, 2 d after dimethoate intoxication the amount
of the cytochrome significantly rose (P<0.05) and
persisted at an elevated level for 2 weeks. This
stimulatory effect of dimethoate was abolished in rats
intoxicated simultaneously with pyrantel (P<0.05 on day
2). One week after intoxication with pyrantel or
dimethoate only, the amount of cytochrome P450
returned to control value.

Cytochrome b5, cytochrome P450:NADPH
reductase and cytochrome b5:NADH reductase. The
changes in the content of cytochrome b5 as well as in the
activity of the analysed reductases (not shown) were
small and statistically not significant, although
corresponded with changes observed for cytochrome
P450.

CYP1A2. The changes in CYP1A2 protein
expression are shown in Fig.1. The effect of pyrantel on
CYP1A2 was minimal and resulted in slight (up to 20%)
and statistically not significant increase in its expression
noted 12 h and 2 d after intoxication. In groups
intoxicated with dimethoate the evident increase in the
CYP1A2 expression was noted as early as 3 h after
intoxication. The expression reached maximum after 2 d
(170% of the control) and then gradually dropped to the
control level on day 14. The observed increase was
statistically significant after 6 h, 12 h and 2 d (P<0.05).
In groups intoxicated with dimethoate and pyrantel
simultaneously the inducible effect was also evident
(statistically significant after 2 d; P<0.05), although
lower than that after intoxication with dimethoate only.
The differences in the level of CYP1A2 expression
between groups D and PD reached the level of statistical
significance 12 h and 2 d after intoxication.

CYP2B1/2. Both pyrantel and dimethoate
exhibited slight inhibitory effect on CYP2B1/2 protein
expression (Fig. 2). In groups intoxicated with
dimethoate (D and PD), this effect (30-40% inhibition)
was statistically significant up to the 6th h after
intoxication. Beginning from day 2 after intoxication,
the levels of CYP2B1/2 expression in all analysed
groups did not differ statistically from the control.

CYP3A1. Pyrantel and dimethoate had
different effects on the expression of CYP3A1 protein
(Fig.3). After pyrantel intoxication, we observed
inhibition of CYP3A1 expression which was evident in
all analysed periods and statistically significant (P<0.05)
6 h and 12 h after intoxication (57% and 43% of control,
respectively). In groups intoxicated with dimethoate the
initial decrease in CYP3A1 expression 3 h and 6 h after
intoxication was followed by evident, although
statistically not significant, increase 12 h and 2 d after
intoxication (12% and 28% of control, respectively). In
groups intoxicated with both xenobiotics simultaneously
the expression of CYP3A1 was generally lower (although the differences were statistically not significant) than in groups intoxicated with dimethoate only.

### Discussion

The cytochrome P450 system is involved in the biotransformation of many different xenobiotics. It has been proved that P450 substrates, or products of their metabolism, may stimulate or inhibit P450 expression and/or activity (9). Our results have shown that the effect of the analysed compounds on the expression of particular components of the cytochrome P450 system was essentially different. After pyrantel administration the total content of cytochrome P450 significantly diminished up to 12 h after the last applied dose, while at the same time the effect of dimethoate was negligible. Two days after pyrantel administration the amount of cytochrome P450 returned to control level, while after dimethoate intoxication rose significantly and persisted at the elevated level for 2 weeks. Such an inducible effect of dimethoate was also noted by Sharma et al. (15) who observed significant increase in P450 level 24 h after a single acute dose (75 and 90 mg/kg b.w.) of dimethoate. In our study this stimulatory effect of dimethoate was abolished in rats intoxicated simultaneously with pyrantel. This indicates, that in the conditions of the performed experiment an interaction between both studied compounds took place.

### Table 1

Total content of cytochrome P450 (nm/mg protein)

<table>
<thead>
<tr>
<th>Time after intoxication</th>
<th>Group</th>
<th>3 h</th>
<th>6 h</th>
<th>12 h</th>
<th>2 d</th>
<th>7 d</th>
<th>14 d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>0.280 ± 0.042</td>
<td>0.382 ± 0.053</td>
<td>0.373 ± 0.061</td>
<td>0.447 ± 0.076</td>
<td>0.503 ± 0.027</td>
<td>0.544 ± 0.086</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>0.526 ± 0.103</td>
<td>0.454 ± 0.067</td>
<td>0.485 ± 0.094</td>
<td>0.702 ± 0.087</td>
<td>0.646 ± 0.103</td>
<td>0.628 ± 0.091</td>
</tr>
<tr>
<td></td>
<td>PD</td>
<td>0.267 ± 0.066</td>
<td>0.257 ± 0.054</td>
<td>0.356 ± 0.076</td>
<td>0.562 ± 0.049</td>
<td>0.537 ± 0.087</td>
<td>0.567 ± 0.073</td>
</tr>
</tbody>
</table>

Control = 0.520 ± 0.046. Statistical significance P<0.05;  x - to the control group; y – to the D group. All data presented as mean ± SD (n=6)

![Fig. 1. Expression level of CYP1A2 protein at different periods after pyrantel and/or dimethoate intoxication. Shown as percentage of control. * - statistically significant to the control; P<0.05](image-url)
Fig. 2. Expression level of CYP2B1/2 protein at different periods after pyrantel and/or dimethoate intoxication. Shown as percentage of control. * - statistically significant to the control; P<0.05.

Fig. 3. Expression level of CYP3A1 protein at different periods after pyrantel and/or dimethoate intoxication. Shown as percentage of control. * - statistically significant to the control; P<0.05.

On the other hand, neither pyrantel nor dimethoate affected significantly any of the electron donors involved in the catalytic cycle of cytochrome P450 (cytochrome b$_5$, cyt.P450: NADPH reductase, cyt.b$_5$:NADH reductase). The cytochrome P450 and the above enzymes formed functional complexes embedded mostly in the membranes of smooth endoplasmic reticulum. The obtained results indicate that during intoxication with pyrantel and/or dimethoate the cytochrome P450 molecule is the most fragile component of the above complex.
The differences between stimulatory/inhibitory properties of the analysed xenobiotics were very evident on the level of cytochrome P450 isoforms. Both pyrantel and dimethoate inhibited the expression of CYP2B1/2. This inhibitory effect was most pronounced during the first several hours after intoxication. Dimethoate was a more potent inhibitor of CYP2B1/2 than pyrantel and there was slight synergistic inhibitory effect of both compounds.

Pyrantel did not significantly affect CYP1A2 expression in any of the analysed time intervals. On the other hand, stimulatory effect of dimethoate was noted as soon as 3 h after intoxication, reaching its maximum 2 d later. The above effect was significantly diminished after simultaneous intoxication with pyrantel supporting the hypothesis that possible interaction between dimethoate and pyrantel may occur.

The most evident differences in the mode of action between dimethoate and pyrantel were noted in case of CYP3A1. Up to the 6th h after intoxication both compounds exhibited inhibitory properties. However, already 12 h after intoxication with dimethoate the level of CYP3A1 was above the control level, reaching its maximum after 2 d. In animals intoxicated with pyrantel the CYP3A1 persisted below the control level throughout all analysed time points. The observed profile of CYP3A1 expression in the studied groups resembles that observed for the total content of cytochrome P450. It seems reasonable that CYP3A1 is the most abundant in rat liver of all studied isoforms.

The mechanisms involved in the inhibition/stimulation of CYP450 expression by pyrantel or dimethoate are not well known. It is suggested that reactive oxygen species generated during the CYP450 catalytic cycle may be one of the important factors. In our previous paper (18) we have shown that intoxication of rats with dimethoate and/or pyrantel decreases the amount of GSH and increases the level of MDA in liver homogenates. The changes in the activities of antioxidative enzymes and increase in MDA content after dimethoate intoxication was also confirmed by others (1,15). Diminished activity of P450 due to lipid peroxidation (or any other mechanism) could lead to the accumulation of its lipid soluble substrates (including dimethoate and pyrantel) in the membranes of smooth endoplasmic reticulum with all negative consequences of this fact. Our results indicate that during the first several hours after intoxication with dimethoate and pyrantel the cytochrome P450 system in the liver does not function properly. However, it must be underline that the biotransforming potential of the liver recovers relatively fast, as 7 d after intoxication most of the parameters (total content of cytochrome P450, expression of CYP isoforms) reach the control level. We have previously shown that during several hours after intoxication the content of both dimethoate and pyrantel in liver homogenates drops rapidly to the residual level. This is probably the time point where the recovery of cell functions involved in the biotransformation of xenobiotics begins. Relatively high total content of cytochrome P450 as well as CYP2B1/2 and CYP3A1 expression observed 2 d after intoxication reflects the top point of this recovery process.

Acknowledgments: This work was supported by KBN grant 3P05D03324.

References