EFFECT OF ORAL ADMINISTRATION OF BI 58 NOWY AND PYRANTEL EMBONATE ON THE ACTIVITY OF SELECTED ANTIOXIDATIVE ENZYMES AND MALONDIALDEHYDE CONTENT IN RATS

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Abstract

The effect of the Bi 58 Nowy (40% dimethoate) and pyrantel embonate, administered separately and in combination, on the activity of superoxide dismutase (SOD) and catalase (CAT) in erythrocytes and on malondialdehyde (MDA) content in rat liver homogenates was investigated. The Bi 58 Nowy and pyrantel embonate were administered per os for 5 d at 1/10 DL50 and for 3 d at 1/5 DL50, respectively. The administration of these compounds caused a significant increase in MDA level in the liver. A larger and more prolonged increase was caused by Bi 58 Nowy. In addition, an elevated activity of SOD (3 h – 14 d) and CAT (2 – 14 d) in erythrocytes was observed, unlike the case of pyrantel embonate. After the combined exposure, slightly stronger changes were observed in the analysed parameters as compared to the separate intoxication by Bi 58 Nowy.

Key words: rats, pyrantel, dimethoate, SOD, CAT, MDA.

The interactions between xenobiotics, introduced to the environment (e.g. pesticides), and commonly applied medicines, thought to be relatively safe, are an important issue, which has yet to be fully explained (17). In order to determine their effect on humans, various experiments have to be conducted with outcomes, which are difficult to predict. The pharmacological and toxicological effects of intoxications can be different than those observed after exposure to separately administered preparations (6, 16). A proper diagnosis of such poisonings would aid in the selection of an appropriate therapy.

It was decided to study this problem due to the wide application of dimethoate (ranked among the top 28 compounds most commonly causing poisoning in humans) in agricultural chemistry (9, 14), and pyrantel embonate in the treatment of helminthiases of the digestive tract in humans and animals (4, 13), and because of possible biochemical interactions between them. The structure and some effects of these compounds indicate that they can induce changes in the biotransformative action in the liver and upset the balance towards intensifying oxidative processes – which has not been the subject of any previous studies (especially in a combined application).

The aim of the study was to analyse the effect of the Bi 58 Nowy and pyrantel embonate, both administered separately or in a combination, on the activity of selected antioxidative enzymes – superoxide dismutase and catalase in erythrocytes and on the content of malondialdehyde in rat liver homogenates (which is considered to be the main indicator of lipid peroxidation).

This study is a part of research relating to the interaction between dimethoate and pyrantel, and their effects on the biotransformative activity of the liver.

Material and Methods

The experiment was conducted on Wistar male rats, with body weights of 180 ± 10 g, divided into 3 experimental groups (I-III) and a control group (C), each with 36 rats. The animals were obtained from the Animal Breeding Centre in Brwinów near Warsaw. During the acclimatisation period they were kept in their standard conditions. Consent from the Local Ethics Committee to conduct the study was obtained.

The experiment was conducted with Bi 58 Nowy (BASF, Germany), which contains 40% of pure dimethoate (0.0-dimethyl-S-methyl-carbamoylmethyl phosphorodithioate), according to the manufacturer’s instructions. Pyrantel embonate was obtained from POLPHARMA (Poland). According to the manufacturer it contains 99.3% of the pure compound.

The animals were fed granulated standard chow “Murigran”. The rats in group I were given pyrantel embonate at 1/5 DL50 (400 mg/kg of b.w.) by a stomach tube for 3 consecutive days. The rats in group II were
given Bi 58 Nowy to the stomach (1/10 DL50 - 37.8 mg/kg of b.w.) for 5 consecutive days. The rats in group III were given both of these compounds at the same doses and periods, with pyrantel embonate administered on days 3, 4 and 5 after the intoxication with Bi 58 Nowy had started.

The rats were decapitated and the biological material (blood, liver) from six randomly selected animals was taken after 3, 6, 12 h and 2, 7, and 14 d from the last intoxication with a particular compound. Blood samples were collected and processed for the isolation of erythrocytes. The livers were homogenized in phosphate buffer, and then homogenates were kept under deep freeze conditions (-70°C) until analysed.

The activity of superoxide dismutase (SOD) in erythrocytes was determined by the kinetic method, with RANSOD analytical kit (RANDOX Lab. Ltd., UK). Quantitative determination of SOD was based on the reaction of a superoxide radical with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT), resulting in a stable red colour; the absorbance was measured at 505 nm. One SOD unit was defined as the amount, which inhibits the reaction with INT by 50%. The activity of SOD was expressed as U/g Hb.

The activity of catalase (CAT) in erythrocytes was determined according to Aebi’s kinetic method (2), taking the advantage of the possibility to trace the constant ratio of hydrogen peroxide decomposition by catalase through recording the drop in absorbance within 15 s at the wavelength of 240 nm. The CAT activity was expressed as U/g Hb. SOD and CAT measurements were taken with a UV/VIS BECKMAN DU 520 spectrophotometer.

Malondialdehyde (MDA) concentration in liver homogenates was determined colourimetrically with BIOXYTECH® MDA-586™ analytical kit (OXIS International Inc., Portland, USA). The method is based on the reaction of N-methyl-2-phenylindole (NMPI) with MDA, resulting in a stable carbocyanine dye – whose maximum absorbance was measured at 586 nm with a UV/VIS Marcel s330 spectrophotometer.

The results were analysed statistically by a one-way analysis of variance (ANOVA) followed by the Newman-Keuls r- test. The results were presented as mean and standard error of mean (±SEM). Differences with P≤0.05 and P≤0.01 were regarded as statistically significant.

Results

The results of SOD and CAT activity in erythrocytes and MDA content in liver homogenates are shown in Tables 1-3.

Following the administration of pyrantel embonate for 3 consecutive days at 1/5 DL50 (group I) after 6 and 12 h, a 11-13% decrease in the SOD activity was observed (Table 1). During the other periods, the enzyme activity fluctuated around the results obtained for the control.

After the intoxication with the Bi 58 Nowy at the doses applied in the experiment for 5 consecutive days (group II), an increase in the SOD activity in erythrocytes was observed as compared to the control; this spanned throughout the experiment. The highest values were observed on days 7 and 14 of the experiment (P≤0.01) (Table 1).

The administration of pyrantel embonate during the intoxication with Bi 58 Nowy (group III) produced a slight increase in the SOD activity (lasting from 12th h to the 14th d) as compared to the animals, which were given only Bi 58 Nowy (group II). However, the increase was statistically insignificant (Table 1).

After treating the rats with pyrantel embonate (group I), the activity of CAT in their erythrocytes was slightly (statistically insignificant - 2-11%) elevated as compared with the control group (Table 2). In the animals, which were given only the Bi 58 Nowy (group II) a significant (P≤0.01) increase in the activity of the enzyme was observed on day 2; this persisted until the end of the experiment (i.e. until day 14). A similar profile of change in the activity of CAT was observed in the rats exposed to mixtures of Bi 58 Nowy and pyrantel embonate (group III), with the increase slightly higher than the value in group II, where the animals were given only Bi 58 Nowy (Table 2).

The MDA content in liver homogenates in all experimental groups and periods, was significantly increased (P≤0.01) as compared to the control. A higher increase in this parameter was observed in the rats from group II, which were given Bi 58 Nowy (Table 3). Soon after the exposure (3 h), a 290% increase in the MDA content was observed; which spanned at this level until day 7, this was followed by a decrease in the parameter. Giving pyrantel embonate to rats (group I), produced a much lower increase in the MDA content (180%) than the application of Bi 58 Nowy, and a decrease in its concentration was observed as early as 6 h after the exposure. The profile of MDA changes in the animals intoxicated with Bi 58 Nowy and pyrantel embonate (group III) was similar to that observed in the group of animals exposed to Bi 58 Nowy. However, after 6 h the increase in MDA concentration in the liver of rats from this group was higher than in those from group II (Table 3).

Discussion

The results of the experiment indicate that the administration of Bi 58 Nowy and pyrantel embonate in the doses applied in the experiment; caused an increase in the activity of SOD and CAT in erythrocytes and MDA content in rat liver homogenates. The increase in erythrocyte SOD activity in the first hours of the experiment (3-12 h) was accompanied by a decrease in CAT activity. The data indicates that the applied dose of the Bi 58 Nowy contributes to the production of reactive oxygen species (ROS), which can stimulate the activity of SOD, thus preventing an increase in oxidative stress.
Table 1
Superoxide dysmutase (SOD) activity in rat erythrocytes after administration of pyrantel embonate, Bi 58 Nowy and Bi 58 Nowy and pyrantel embonate expressed as U/g Hb^a

<table>
<thead>
<tr>
<th>Group of animals (n = 6)</th>
<th>Time after intoxication</th>
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<tr>
<td></td>
<td>3 h</td>
<td>6 h</td>
<td>12 h</td>
<td>2 d</td>
<td>7 d</td>
<td>14 d</td>
</tr>
<tr>
<td>Control (C)</td>
<td>1537.44/±54.90</td>
<td>1529.41/±59.72</td>
<td>1556.88/±90.36</td>
<td>1555.78/±100.96</td>
<td>1454.90/±65.39</td>
<td>1449.05/±69.45</td>
</tr>
<tr>
<td>Pyrantel embonate (I)</td>
<td>1588.66/±56.68</td>
<td>1320.43/±99.19</td>
<td>1374.82/±58.97</td>
<td>1505.92/±71.90</td>
<td>1480.92/±44.03</td>
<td>1484.05/±100.30</td>
</tr>
<tr>
<td>Bi 58 Nowy (II)</td>
<td>1773.94/±78.37</td>
<td>1938.36/±64.97</td>
<td>1775.13/±115.95</td>
<td>1680.08/±84.92</td>
<td>1953.46/±103.25</td>
<td>2120.07/±64.02</td>
</tr>
<tr>
<td>Bi 58 Nowy +</td>
<td>1570.48/±47.31</td>
<td>1818.24/±105.91</td>
<td>1803.67/±88.16</td>
<td>1931.39/±166.32</td>
<td>2055.11/±34.20</td>
<td>2154.81/±72.06</td>
</tr>
<tr>
<td>Pyrantel embonate (III)</td>
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<tr>
<td>Statistical differences</td>
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<tr>
<td>P_I&lt;0.05</td>
<td>P_C-I&lt;0.05</td>
<td>P_I-II&lt;0.05</td>
<td>P_C-I&lt;0.01</td>
<td>P_C-I&lt;0.05</td>
<td>P_I-II&lt;0.01</td>
<td>P_C-II&lt;0.01</td>
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<td>P_I&lt;0.01</td>
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a – values expressed as mean ±SEM of 6 rats.

Table 2
Catalase (CAT) activity in rat erythrocytes after administration of pyrantel embonate, Bi 58 Nowy and Bi 58 Nowy and pyrantel embonate expressed as U/g Hb^a

<table>
<thead>
<tr>
<th>Group of animals (n = 6)</th>
<th>Time after intoxication</th>
<th></th>
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<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>3 h</td>
<td>6 h</td>
<td>12 h</td>
<td>2 d</td>
<td>7 d</td>
</tr>
<tr>
<td>Control (C)</td>
<td>537.27/±11.12</td>
<td>550.51/±21.36</td>
<td>498.08/±17.83</td>
<td>568.16/±31.08</td>
<td>542.71/±5.69</td>
</tr>
<tr>
<td>Pyrantel embonate (I)</td>
<td>598.35/±39.15</td>
<td>576.62/±28.15</td>
<td>520.54/±19.34</td>
<td>553.35/±25.16</td>
<td>556.66/±23.25</td>
</tr>
<tr>
<td>Bi 58 Nowy (II)</td>
<td>506.61/±30.44</td>
<td>504.60/±18.35</td>
<td>453.59/±21.55</td>
<td>636.92/±17.28</td>
<td>646.13/±26.05</td>
</tr>
<tr>
<td>Bi 58 Nowy +</td>
<td>522.34/±38.94</td>
<td>525.51/±18.89</td>
<td>474.65/±21.85</td>
<td>669.01/±27.73</td>
<td>667.79/±21.93</td>
</tr>
<tr>
<td>Pyrantel embonate (III)</td>
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<tr>
<td>Statistical differences</td>
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<tr>
<td>P_C-I&lt;0.05</td>
<td>P_I-II&lt;0.05</td>
<td>P_I&lt;0.01</td>
<td>P_C-I&lt;0.05</td>
<td>P_I-II&lt;0.01</td>
<td>P_I-II&lt;0.01</td>
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<td>P_I&lt;0.01</td>
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</table>

Explanations as in Table 1.
Table 3
Malondialdehyde (MDA) content in rat liver after administration of pyrantel embonate, Bi 58 Nowy and Bi 58 Nowy and pyrantel embonate expressed as µM/g fresh tissue

<table>
<thead>
<tr>
<th>Group of animals</th>
<th>(n = 6)</th>
<th>Time after intoxication</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3 h</td>
</tr>
<tr>
<td>Control (C)</td>
<td></td>
<td>4.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.33/</td>
</tr>
<tr>
<td>Pyrantel embonate (I)</td>
<td></td>
<td>11.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.75/</td>
</tr>
<tr>
<td>Bi 58 Nowy (II)</td>
<td></td>
<td>16.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±1.79/</td>
</tr>
<tr>
<td>Bi 58 Nowy + Pyrantel embonate (III)</td>
<td></td>
<td>15.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±2.14/</td>
</tr>
</tbody>
</table>

Statistical differences
- P_{I,III} ≤ 0.05
- P_{C,II,III} ≤ 0.01
- P_{C,II,III} ≤ 0.01
- P_{C,II,III} ≤ 0.01
- P_{C,II,III} ≤ 0.01
- P_{C,II,III} ≤ 0.01
- P_{C,III} ≤ 0.01
- P_{C,III} ≤ 0.01
- P_{C,III} ≤ 0.01
- P_{C,III} ≤ 0.01

Explanations as in Table 1.

The increase in the enzymes activity can be explained as a defensive reaction of red blood cells to the oxidative effect of ROS, which results from the oxidative stress caused by the administration of the compounds. This is confirmed by the studies of other authors who have pointed out that phosphoroorganic insecticides usually induce oxidative stress and generate free radicals (1, 3, 10).

The data obtained from the literature indicate that, dimethoate causes an increase in the activity of SOD and CAT in some tissues. Sharma et al. (11, 12) gave rats various doses of dimethoate per os and observed increased activity of SOD and CAT in the liver and brain, which was dependent on the applied dose of the insecticide. The highest increase in the SOD activity after a single dose (90 mg/kg of b.w.) of the toxic compound was observed in the liver, and in the case of CAT activity in the brain (75 mg/kg of b.w.) (11). The highest increase in the activity of the enzymes in the liver and brain, after the 30-d long intoxication with dimethoate was observed after administration at the dose of 30 mg/kg of b.w. (i.e. 1/10 DL_{50}) (12). The increase is explained by the authors by activating the defensive mechanisms against the pro-oxidative effects of the insecticide; the increase in the oxidative stress is explained by becoming addicted to the dimethoate dose.

The significant increase in the activity of SOD and CAT in rat erythrocytes observed in this study after administering Bi 58 Nowy and pyrantel embonate, was similar to that observed by John et al. (6), both after separate and combined application of dimethoate (at 0.03 mg/kg of b.w.) and malathion (at 0.13 mg/kg of b.w.).

Malondialdehyde content in tissues is considered to be the biomarker of oxidative stress of the organism. Its concentration was found to be increased, e.g. after administration of phosphoroorganic compounds (5, 15). In this study, the MDA content in the liver was also found to be significantly increased after the administration of both Bi 58 Nowy and pyrantel embonate. The increase was higher and more prolonged after the application of the phosphoroorganic insecticide. During the first hours after exposure, a high concentration of MDA in liver homogenates was accompanied by elevated activity of SOD and decreased activity of CAT in erythrocytes. This could indicate a lack of balance between the pro- and anti-oxidative processes and intensification of changes towards pro-oxidative processes.

A similar increase in MDA concentration in the liver to that recorded in this study after the administration of Bi 58 Nowy was observed by Sharma et al. (12), in their study on rats intoxicated for 30 d with dimethoate at a dose of 6 and 30 mg/kg of b.w. Increased concentrations of MDA after administration of dimethoate were also recorded in the studies on other animal species. Maiti and Kar (8) gave dimethoate to mice for 30 d at a dose of 2, 4 and 8 mg/kg of b.w., and observed intensified lipid peroxidation, which was measured as the amount of MDA produced in the liver. The highest concentration of MDA was observed after the dose of 8 mg/kg of b.w. In a study, in which chickens were given the Rogor preparation (30% dimethoate); Maiti et al. (7) observed a significant increase in MDA concentration in the kidneys and liver. The strongest increase also took place after a dose of 8
mg/kg of b.w. The authors point out that dimethoate stimulates the production of free radicals, thus acting as a hepatotoxic and nephrotoxic agent, which results in increasing of the concentration of MDA in the liver and kidneys.

When rats were exposed to the combined action of Bi 58 Nowy and pyrantel embonate, the profile of changes in MDA concentration in the liver was similar to that observed after administration of the insecticide separately. Pyrantel embonate increased MDA concentration in liver homogenates only slightly as compared to the values observed in the group of animals exposed to the Bi 58 Nowy. This phenomenon was not observed by John et al. (6) in their study on rats intoxicated with dimethoate and malathion simultaneously. The differences in MDA concentration between those compounds may be linked to their structure and dose.

It can be concluded that administration of Bi 58 Nowy and pyrantel embonate in the applied doses induced significant changes in SOD and CAT activities in erythrocytes and MDA concentrations in rat liver homogenates.

During the initial hours after the exposure, a high concentration of MDA in the liver was accompanied by increased SOD activity and decreased CAT activity in erythrocytes. This could indicate a lack of balance between pro- and anti-oxidative processes during the period of the study and intensification of changes towards the pro-oxidative processes. A significant increase in SOD and CAT in erythrocytes was observed between day 2 and 14 after administration of Bi 58 Nowy, which was not the case after the application of pyrantel embonate.

The compounds applied in the experiment caused a significant increase in MDA concentration in rat liver as compared to the control group. A larger and more prolonged increase was observed after administration of Bi 58 Nowy.

The profile of parameter changes in combined intoxication was similar to that observed after the application of Bi 58 Nowy, but the changes were more intense.

References