Biochemical Changes as Biomarkers of Pyrethroid Toxicity in Rats

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Abstract: Biochemical Changes as Biomarkers of Pyrethroid Toxicity in Rats: Tong Jian, et al. Department of Public Health, Suzhou Medical College—Biochemical changes reflecting toxic effects of two pyrethroid pesticides were pursued under experimental conditions of single and multiple exposures. Two pesticides, fenvalerate and cypermethrin, were administered to rats, and biochemical indices such as levels of enzymes and proteins in blood and bronchoalveolar lavage fluid (BALF) were measured. Results indicated that with multiple oral administration of cypermethrin to rats, a significant decrease in blood cholinesterase (ChE) activity could be observed after 1 wk of daily treatment, which suggested a secondary and indirect response. On the other hand, lung damage induced by fenvalerate seemed to be an acute effect, since all the biochemical effects measured in the present study appeared within 1 d after the single exposure, and could no longer be detected 1 wk later. These findings indicated that biochemical changes in biological samples could be used as biomarkers, which in turn may provide a supplementary means for the recognition of potential adverse effects of the pesticides as well as for the protection of sensitive individuals in an occupational context. (J Occup Health 1996; 38: 54–56)

Key words: Biochemical effects, Pyrethroid pesticides, Biomarkers

The application of biomarkers, linked to toxicity or the risk assessment process, has been developed in recent years to provide a more rational means in toxicological studies. Among biomarkers of various types, biochemical changes are most widely used for assessing adverse health effects in exposure to harmful agents from any source.

The toxic actions of pyrethroid pesticides have been studied for years by detecting electro-physiological changes in transductive process of the transmitters in the nervous system, in consequence leading to injury to ion channels of the biological membrane, etc. Nevertheless, some work on biochemical effects of the pesticides began to be documented recently, which suggested that biological indicators could also be used for monitoring and screening the toxic effects of pyrethroids. In most of the studies, however, treatment of animals was performed only once, and longer effects following repeated exposures, which were of much more significance in toxicological studies, were almost uninvestigated.

This paper deals with adverse effects on rats by two pyrethroid pesticides: Cypermethrin and fenvalerate, under experimental conditions involving one and multiple exposures. Attention was paid to the in vivo toxic effects of the pesticides, with observations on the time course of levels of the biochemical indicators such as enzyme activities of cholinesterase (ChE), malate dehydrogenase (MDH) and lactate dehydrogenase (LDH).

Materials and Methods

Pesticides: Cypermethrin and fenvalerate (90.3% v/v) were provided by the Jiangsu Institute of Agriculture Science and Hormone Research Centre (Suzhou, China), with the oral LD50 previously obtained from rats at 250 mg/kg and 70 mg/kg, respectively. Cypermethrin was diluted with high quality rapeseed oil and fenvalerate with 0.9% NaCl solution before use.

Animal treatment: In the cypermethrin experiment, male Wistar rats of 180–250 g body weight were randomly divided into groups of 10. The rats were orally treated with the pesticide daily, at a dose of 1/3 LD50 (82 mg/kg) and 1/10 LD50 (24 mg/kg) for each group. The daily treatment of the high dose group (1/3 LD50) continued for 15 d until the cumulative dosage reached 5 LD50, while the treatment of the low dose group (1/10 LD50)
lasted for 10 d with a cumulative dosage of 1 LD50.

In the fenvalerate experiment, groups of 10 male Wistar rats of 180-250 g body weight were instilled intratracheally with a 1/15 LD50 (4.7 mg/kg) dose, and sacrificed at d 1, wk 1, wk 2 and wk 3 separately after the treatment. The control group (10 rats) were given 0.9% NaCl solution and sacrificed at d 1.

**Biochemical measurements:** From rats that received cypermethrin, blood samples were taken by cutting off the tail for each group before and after the treatment once a week over 3 wk. The samples taken before the treatment were used as the control, while those taken 1 d after the first daily treatment as d 1, 1 wk after the first daily treatment as wk 1, and so forth. Determination of ChE activity in the serum and erythrocytes was carried out by a modified Ach/DTNB (di-tetrazolium nitroblue) procedure as reported previously. In rats treated with fenvalerate, MDH and LDH activities, and amounts of albumin (ALB) and total protein (TP) in the bronchoalveolar lavage fluid (BALF) were measured at the time the rats were sacrificed. The BALF was obtained by washing down 3 ml of 1% saline into the bronchoalveolar tract and then drawing out, repeating three times with a total volume of 9 ml.

**Statistical analysis:** Statistical analysis was made by the paired Student’s t-test for the cypermethrin data, and the unpaired Student’s t-test for the fenvalerate data.

### Results

**Effects of cypermethrin on serum and erythrocyte ChE activities:** All the samples taken 1 d after the first treatment for the measurements of serum and erythrocyte ChE activity showed insignificant fluctuations (Table 1). In experimental group of both 1/3 and 1/10 LD50 of the pesticide, serum ChE activity 1 wk after the first treatment was found significantly lower than that of the control. and continued to decrease during the second v. while the daily treatment was going on. For the erythrocyte ChE, the time course of the activity changed in a pattern similar to that of the serum ChE in the 1/3 LD50 group, but in the 1/10 LD50 group, a significant decrease in the erythrocyte ChE activity first occurred 2 wk after the initial treatment.

It can also be seen from Table 1 that the inhibitions of both erythrocyte and serum ChE disappeared during the third week in which the treatment had stopped, and the activities finally returned to the control level.

**Effects of fenvalerate on biochemical changes in the BALF:** All the levels of MDH, LDH, ALB and TP showed significant changes 1 d after the exposure, as compared to the control group. Most of the changes, however, recovered to the basal level in 1 wk, and eventually returned to the control in 2 or 3 wk (Table 2).

<table>
<thead>
<tr>
<th>Time course</th>
<th>Serum ChE activity</th>
<th>Erythrocyte ChE activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1/3 LD50 (82 mg/kg)</td>
<td>1/10 LD50 (24 mg/kg)</td>
</tr>
<tr>
<td></td>
<td>1/3 LD50 (82 mg/kg)</td>
<td>1/10 LD50 (24 mg/kg)</td>
</tr>
<tr>
<td>Before treatment</td>
<td>0.40±0.03</td>
<td>0.53±0.01</td>
</tr>
<tr>
<td>d 1</td>
<td>0.38±0.05</td>
<td>0.50±0.07</td>
</tr>
<tr>
<td>wk 1</td>
<td>0.27±0.06*</td>
<td>0.35±0.04*</td>
</tr>
<tr>
<td>wk 2</td>
<td>0.12±0.03*</td>
<td>0.32±0.06*</td>
</tr>
<tr>
<td>wk 3</td>
<td>0.43±0.04</td>
<td>0.59±0.12</td>
</tr>
</tbody>
</table>

*P<0.01. Values are the mean±SD (μM). 10 animals in each group.

<table>
<thead>
<tr>
<th>Time course</th>
<th>LDH (IU/l)</th>
<th>MDH (IU/l)</th>
<th>ALB (g/l)</th>
<th>TP (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>198±114</td>
<td>154±47</td>
<td>0.10±0.02</td>
<td>0.37±0.12</td>
</tr>
<tr>
<td>d 1</td>
<td>480±162*</td>
<td>227±32*</td>
<td>0.37±0.15*</td>
<td>1.28±0.40*</td>
</tr>
<tr>
<td>wk 1</td>
<td>180±30</td>
<td>156±18</td>
<td>0.14±0.05</td>
<td>0.89±0.29*</td>
</tr>
<tr>
<td>wk 2</td>
<td>204±90</td>
<td>179±45</td>
<td>0.12±0.04</td>
<td>0.41±0.11</td>
</tr>
<tr>
<td>wk 3</td>
<td>234±90</td>
<td>165±31</td>
<td>0.12±0.04</td>
<td>0.34±0.08</td>
</tr>
</tbody>
</table>

*P<0.01. Values are the mean±SD. 10 animals in each group.

Discussion

It has been believed that pyrethroid pesticides do not inhibit ChE which is the toxic target of organophosphorus pesticides. Many of the earlier reports claimed that the activity of ChE was not obviously affected by the pyrethroid treatment in either in vivo or in vitro experiments. A few of later reports, however, mentioned positive findings in changes in the ChE activity after pyrethroid exposure. In one experiment, although no effect was detected 30 min after the treatment, a significant decrease in erythrocyte and serum ChE activity was clearly observed 13 d later. This discrepancy suggested that a time course study was necessary to investigate the inhibitory effect of pyrethroids on ChE.

Our results with multiple treatment of rats with cypermethrin indicate that the interval between the pesticide treatment and the time at which samples are taken for ChE measurement makes a great difference in the effects of the pyrethroid on ChE. When the measurement was conducted 1 d after a single treatment, the ChE activities showed only a small shift with no statistical difference, but when the interval was long enough (e.g. 1 wk), a significant decrease in ChE activities could then be perceived after multiple daily treatments. This interval-related response suggests a secondary and indirect effect of the pesticide rather than an acute and direct one.

Fenvalerate is another pyrethroid pesticide widely used in agriculture and daily life. Although its acute toxicity to animals is moderate, with toxic processes mainly occurring in the central nervous system, it was suspected recently that it may also impair the biochemical components once being absorbed by inhalation.

The damage to the lung induced by fenvalerate seemed to be an acute effect, since all the biochemical changes in the BALF appeared 1 d after the exposure, and could not be detected 1 wk later, except TP which might result from the inflammatory response of the lung tissue. The precise mechanism of the damage is not yet fully understood. Factors like the in vivo absorption, distribution, metabolic dynamics and toxic targets of the pesticide may all be involved.

Biomarkers can be used to confirm the exposure of individuals in a population to a particular substance. Quantitative assessment of biomarkers may facilitate the determination of dose-response and time-response relationships. In an occupational context, biomarkers can be applied as a supplementary means for reviewing the adequacy of protective measures, including work practices and working conditions.

The use of biomarkers reflecting the susceptibility of animals to pyrethroid pesticides provides an opportunity to determine the degree of biochemical damage to them. Biomarkers may be useful and helpful, not only in understanding the potential adverse health effects of the pesticides, but also in monitoring the effects on workers.

References