Blood and tissue levels of diazinon in rabbit following a subacute dermal exposure to incremental doses

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Abstract:
BACKGROUND: Uncontrolled application of diazinon (DZN) can cause environmental contamination and adverse health effects on humans or animals. OBJECTIVES: This study aimed to investigate the toxic effects and the level of DZN in serum and tissues of rabbits following a sub acute dermal exposure to toxicant. METHODS: Different doses of DZN were applied daily to New Zealand rabbits through the ear skin in incremental doses for 4 weeks. Blood samples were collected at the beginning and the end of each dose-week period. Tissue samples were collected from brain, muscle, kidney and liver on day 28, after euthanizing the rabbits. DZN contents of the blood and tissue samples were measured using a reversed phase HPLC system. RESULTS: Clinical observations indicated signs of toxicity in the animals exposed to DZN as shown by diarrhea and body weight loss from day twenty. The level of DZN in the blood elevated with enhancing exposure time and reached the highest level at the end of the fourth week (0.620±0.26ppm). The highest level of DZN was found in the brain tissue (0341±0.015 ppm). CONCLUSIONS: The results of this study revealed the tissue accumulation and subsequent toxic effects of DZN following the subacute dermal exposure to the toxicant. It suggests that the determination of the toxicant level in the serum or tissue can be a monitoring method for the detection of the contamination rate.

Introduction

Organophosphorus (OP) compounds are phosphate esters extensively used to control different pests, parasites and fungi in veterinary medicine or to kill weeds in agriculture. Diazinon (DZN) as an organophosphorus compound was first developed as an insecticide, acaricide, and nematicide, and is currently used as a wide spectrum contact pesticide in many countries against insects and other parasites living on the pets, farm animals, crops and ornamental plants (Cupta, 2006; Jeyaratnam & Maroni, 1994). Extensive and uncontrolled application of toxicants may lead to the contamination of the environment and thus causes serious health problems. Noticeable accumulations of these compounds have been detected in marine and non-marine organisms, soil, fruits and vegetable field crops (Hayes, 1980; Larkin et al., 2000).

Diazinon exerts its toxic effects by the inhibition of acetylcholinesterase (AChE), an enzyme necessary for the function of the central and peripheral nervous systems. It irreversibly binds to AChE and inhibits the hydrolysis of the acetylcholine (ACh) in cholinergic synapses and neuromuscular junctions. This can result in the enhanced accumulation of ACh at cholinergic receptors leading to overstimulation of nerves and muscles. High level of DZN exposure can lead to neurotoxic effects manifested by bronchoconstriction, increased secretion, diarrhea, hypoten-
sion, tachycardia, muscular twitching, cramping, drowsiness, headaches, ataxia, seizure and respiratory depression (Bean et al., 2005; Dahlgreen et al., 2004; Garfitt et al., 2002; Davies & Holub, 1983).

Diazinon, as an important compound, has been chosen for this investigation because of its worldwide usage and its contaminating influence on the environment. It dissolves well in organic solvents and is readily absorbed through different exposure routes including inhalation, skin and oral administration. Although the common route of DZN exposure is through the ingestion of contaminated food or drinking water, its absorption via other ways including skin or inhalation is of major concern in animals or human populations including farmers, workers, or commercial applicators involved in manufacturing or using toxicants (James et al., 1983; Lenhart & Kawamoto, 1994; Williams et al., 1987). The DZN absorption rate through epidermis depends on the temperature of the place of contact, the water content of the dermis and the integrity of the skin (Ecobichon, 2001; Garcia-Repetto et al., 1994; Frank et al., 1991).

It is readily absorbed in the body once exposed and finds its ways through the blood-brain barriers into the brain. High accumulation of DZN in high-fat tissues such as brain indicates the lipophilic nature of this compound (Ecobichon, 2001; Yang et al., 2000). Ingestion of foods or drinking water contaminated with a small amount of DZN is reported to be the most important route of exposure for the general population who are not dealing with the extensive application of DZN. However, people living near the agricultural areas where DZN is extensively used, or the farmers and workers involved in the production, distribution and marketing of DZN may be at an increasing risk of exposure to a higher level of the toxicant through other routes, including skin. Diazinon toxicity via dermal contacts may constitute a small number of the total exposures, but constant dermal exposure may lead to the accumulation of the toxicant in different tissues. This can result in chronic toxicity and subsequent biochemical and physiological alterations (Garfitt et al., 2002). However, the data regarding the accumulation of the toxicant in human or animal tissues and the consequent adverse health effects associated with dermal contact are limited. The current study was designed to examine the toxic effects of DZN and the levels of this toxicant in the blood and different tissues following a subacute (28-day period) dermal (through ear skin) exposure of rabbits to incremental doses.

Materials and Methods

Animals: Twelve white New Zealand rabbits, each one weighing around 1800±100 g, were used in this study. The animals were kept in a room with a temperature of 20±3°C at regular light/dark cycles, and they had free access to food and water. They were maintained under the standard conditions and all the procedures performed on animals followed the institutional ethical guides established by the Institutional Care and Use of Laboratory Animal Committee.

Study Design: The rabbits were randomly divided into two experimental groups including test and control. The control animals were only solvent treated, but the animals in the test group were exposed to DZN through dermal route (ear skin) in an accumulating dose-week manner for four weeks. The exposure was carried out by daily doses of 50, 100, 250 and 400 mg/kg of DZN on days 1, 8, 15 and 22 during four weeks (each dose throughout a week). All the contacts with diazinon were made only through the skin of one ear of the animals. The toxicant was applied as spray and efforts were made to keep the surface of other parts of the body free of contamination. One puff of sprayer was adjusted to supply 25 mg/kg diazinon on ear skin and repeated spraying was performed to reach the proposed doses of the toxicant. Continuous observations and physical examinations were made to assess the animals' health conditions.

Sampling: The blood samples were collected at different time intervals and the tissue samples were collected at the end of the experiment. The blood samples (3 mL) were drawn from each rabbit via ear marginal veins before exposure to DNZ and at the end of each dose-week period (on days 0, 7, 14, 21 and 28). The bloods were centrifuged; the sera were separated and kept at -20°C until analysis. Tissue samples were taken from the brain, liver, kidney and the muscle of each rabbit on day 28. The animals were euthanized and tissue specimens were collected from liver, kidney, muscle and brain. The tissues were wrapped in aluminum foils and were kept at -20°C until...
Extraction and determination of DZN: The extraction and HPLC procedures used to measure the diazinon contents of the samples were based on the method developed by Abou-Donia and Abu-Qare (2001) with a few modifications. The serum was thawed and the extraction was started by dilution of 1 mL serum into 4 mL distilled water. Diluted samples were passed through pre-conditioned Sep-Pak C18 cartridges with 5 mL acetonitrile plus 3 mL distilled water. The cartridge was eluted with 1 mL acetonitrile and the collected elution was used for HPLC analysis after filtering through 0.25 micron disk filters. The freeze-dried tissues were also thawed and homogenized using a standard homogenizer (KINEMATICA AG Dispersing and Mixing Technology). Five hundred mg homogenized tissue was suspended into a solution containing 1 mL HCl and 2 mL acetonitrile. The suspension was shaken for 30 minutes and then dried at 40°C using incubation with nitrogen flow. The next steps of the extraction were the same as those performed on the serum samples using Sep-Pak C18 cartridges.

A reversed-phase HPLC system was used to measure the concentration of diazinon in serum and tissue samples. The system was equipped with a C18 column (300×5mm, particle size: 5µm), Wellchrom K-1001 pump, Triathlon auto-sampler and Smartline UV detector set at 254 nm (all from KNAUER, Germany). The mobile phase consisted of a mixture of acetonitrile and water (78:22%) adjusted at pH=3 and the flow rate was 1 mL/min. A fifty µL of eluted sample prepared from extraction of tissue or blood sample was injected into the HPLC pump.

Statistical analysis: All data are expressed as the mean ± SEM obtained from six animals tested in each group. Data were analyzed by one-way analysis of variance (ANOVA) using SPSS software. The significance of the differences between test and control group was tested by Tukey post-hoc test. A p-value less than 0.05 were considered statistically significant.

Results

Toxic effects: Clinical signs indicated that diazinon induced toxic effects in the group exposed to toxicant through ear skins. The toxic effects of DZN was shown by a significant decrease in body weight (p<0.001) and the occurrence of diarrhea in the exposed animals. However, none of the animals in the control group showed weight loss or diarrhea. While the animals in control group showed a significant (p<0.01) weight gain at the end of experiment (from 1550±73 to 1885±75), there were a significant weight loss (p<0.001) in DZN-exposed animals (1580±74 to 1080±65). The occurrence of diarrhea and subsequent weight loss started from day 20 of the treatment and continued to the end of the experiments.

DZN replaced by diazinon: The typical chromatographs obtained from the analysis of the blank sample, the standard solution and the serum of a rabbit exposed to diazinon is shown in figure 1. It was found that the increased concentrations of DZN in the serum of exposed animals were dependent on both time and dose of the exposure. The levels of diazinon in the serum of exposed rabbits at different times of sampling are summarized in table 1. As this table shows, the highest concentrations of DZN (0.620±0.265 ppm) were shown at the end of the fourth week in which the rabbits were exposed to 400 ppm diazinon. A gradual increase of the level of DZN in the serum of the rabbits exposed to a sub acute (28

<table>
<thead>
<tr>
<th>Sampling time (day)</th>
<th>Mean diazinon (ppm)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>0.183</td>
<td>0.074</td>
</tr>
<tr>
<td>14</td>
<td>0.356</td>
<td>0.100</td>
</tr>
<tr>
<td>21</td>
<td>0.502</td>
<td>0.240</td>
</tr>
<tr>
<td>28</td>
<td>0.620</td>
<td>0.260</td>
</tr>
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</table>

Table 1. The level of diazinon (mean±SEM) detected in the serum of DZN-exposed rabbits exposed to toxicant through dermal route (ear skin) in an accumulating dose-week manner for 4 weeks. SEM: Standard errors of mean, ppm: Particle per million.

Table 2. The mean level of diazinon (Mean±SEM) detected in different tissues of rabbits exposed to toxicant through ear skin in an accumulating dose-week manner for 4 weeks. SEM: Standard errors of mean.

<table>
<thead>
<tr>
<th>Tissue sample</th>
<th>Mean of diazinon (µg/g of tissues)</th>
<th>SEM (N=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>0.341</td>
<td>0.015</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.203</td>
<td>0.073</td>
</tr>
<tr>
<td>Liver</td>
<td>0.135</td>
<td>0.057</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.050</td>
<td>0.007</td>
</tr>
</tbody>
</table>
(72x182) days period) dermal (ear skin) incremental doses of toxicant is depicted in figure 2. The level of DZN in the tissue samples collected from different organs (brain, kidney, liver, muscle) is shown in table 2. As this table indicates the highest concentration of diazinon residues was obtained in the brain (0.341±0.015 ppm), but the lowest level was detected in the muscles (0.050±0.007 ppm). The average levels of diazinon in the kidney and the liver were 0.203±0.073, and 0.135±0.057 ppm, respectively.

Discussion

Occupational exposures to DZN may occur via dermal contact or inhalation in the workplace where the toxicant is manufactured or applied on animals or

Figure 1. Typical chromatographs obtained from analysis of blank sample (A), standard solution of diazinon (B), and the serum of a rabbit exposed to diazinon (C). DZN = diazinon peak.
field crops. Populations living within or near areas of heavy DZN application would have increased risk of exposure to large levels of the compound through dermal contact with contaminated agents. The main aim of the current study was to evaluate diazinon toxicity and its levels in the serum and four main tissues of rabbits following a sub acute (28 day period) dermal (ear skin) exposures of rabbit to incremental doses. Application of the toxicant to the animals was in cumulative doses with gradual elevation of dosing during four continuous weeks. This means that during the period of the study a daily exposure of rabbits to DZN has been performed with a stepwise elevation in applied doses of toxicant. Our findings showed that a stepwise increase in the applied doses of DZN was associated with a gradual increase in serum levels of the toxicant from 0.183 ppm (on day 7) to 0.620 ppm (on day 28). Traces of DZN residues in different tissue showed the highest accumulation of the compound in the brain (0.341±0.015 ppm), but its level in the serum of the treated animals at the end of the experiment was higher than the tissues. The toxic effects of DZN were shown by a significant decrease in the body weight and diarrhea occurring on day 20 when the serum level of the toxicant was 0.502ppm.

The increased accumulation of DZN in the blood of the exposed animals shows the readily absorption of diazinon through dermis and its potential to access into different tissues. Increased time of exposure was associated with enhanced concentration of the toxicant in the circulation. Furthermore, the stepwise increases in the exposure doses through four continuous weeks lead to more absorption and higher concentration of the toxicant in the blood. The high concentration of DZN in the brain could be due to the lipophilic property of the compound that permits its readily influx into the nervous system and causes neurotoxic effects in animals (Garfitt et al., 2002; Tomokuni et al., 1985; Wester et al., 1993; Wu et al., 1996). The kidney was the second tissue (in grade) for toxicant accumulation with the level of 0.203 ±0.073 ppm in the animals exposed to diazinon. The high concentration of diazinon in the renal tissue samples was predictable because of the increased renal blood flow, and being the main organ for excretion of DZN and its metabolites. The liver tissue had the third rank in diazinon content (0.135±0.057 ppm) compared to the brain and kidney. Muscle tissues with diazinon content of 0.05±0.007 ppm had the lowest concentration rate of DZN compared to the other tissues, which was also predictable because of the lowest blood supply and lipid content of the muscles. These findings may be important with regard to the issues of toxicant residues in the meat of the food producing animals.

The risks of the absorption of diazinon through dermal contacts, its accumulation in different tissues including CNS and probably the incidence of toxicity can be an important way of exposure (Hayes et al., 1980; Timchalk, 2001; Wester et al., 1993). However, there is limited information to show the feature of toxicity induced through this way of exposure. Obscure but continuous exposure of many workers in the marketing or distributing places can be the second form of exposure to the toxicant. General population may also be contaminated by DZN through consumption or dermal exposure to materials being contaminated with the toxicant, including meat, dairy products, vegetables, fruits and so on (Tomokuni et al., 1985; Wu et al., 1996). Chronic toxicity may occur due to continuous contamination of the animal through different routes of exposure including skin contact (Atlanta, 1999). Thus, determination of diazinon residues in serum or tissue samples (if possible) of animals or people who are in the continuous and close contact with the toxicant can be a critical assay for predicting or recognizing relative toxicity.

The clinical signs of toxicity due to diazinon
appeared after 20 days of dermal contacts with the toxicant. Significant weight loss and incidence of diarrhea was the most important signs of toxicity in exposed animals in the present study. It seems that the concentration 0.50±0.24 ppm diazinon in serum of rabbit was adequate to induce toxicity in rabbits. This level of DZN was detected at the third week of exposure when the animals were exposed with a cumulative dose of 50, 150 and 250 mg/kg toxicant. The induction of toxicity by DZN depends on different factors including absorption rate, animal species, contact routes and time of exposure. There is evidence that the rat is more sensitive than the other mammalian species with an acute oral LD50 of 224 mg DZN/kg body weight (Eisler, 1986; Machin et al., 1975), and mammals are shown to be more resistant to DZN-induced acute oral toxicity compared to the birds (Egyed, 1974; Eisler, 1986; Schafer et al., 1983). An inhalation-induced toxicity study showed that 27.2 mg of diazinon/l of air caused 50% death in rabbits exposed to toxicant for 4 hours (Anon, 1972).

Diazinon-induced toxicity can occur in human or animals if they are dermally exposed to the toxicant and the feature of toxicity is largely dependent on the animal species, duration of exposure, differences in purity of formulation, type of solvent, and area of exposed skin. In an experimental study in which the DZN was applied to the shaved dermal area of rats of both sexes, the LD50 values were 900 and 455 mg/kg for males and females, respectively. The use of an occlusive dressing after dermal application can increase dermal toxicity because of enhanced sweating and dermal absorption (Gaines, 1960). Similarly, when 3 subsequent doses of 275, 550 and 1100 mg of DZN suspension were applied to the guinea pigs skin, it led to the animals’ deaths after 9 days. Furthermore, following the application of ascending doses of 300 to 900 mg/kg DZN on the shaved skin of the rabbits for 7 weeks, the first signs of toxicity appeared at 700 mg/kg and death occurred at the dose of 900 mg/kg (EPA, 1990). In the present study, we found that when the serum level of the stepwise enhancement doses of DZN from 50 to 250 mg/kg reached 0.50±0.24 ppm, the clinical signs of toxicity can be observed in the exposed animals. The differences in the results of these studies may be due to the different ways of dermal exposure to DZN, and it seems that the application of DZN in spray form is actually closer to the way of contamination in occupational contact.

In conclusion, in a sub acute dermal exposure of rabbit to incremental doses of diazinon, the toxic effect occurred on day 20 when the level of toxicant in the serum was 0.50±0.24 ppm. The highest accumulation of DZN was in the brain, but its level in the serum of the exposed animals at the end of the study was higher than the tissue levels. The results of this study further documented the possibility of DZN accumulation and subsequent toxic effects through chronic dermal exposure. It also suggested that plasma concentration of DZN can be an appropriate method to evaluate the contamination rate and the prediction of the likely toxic effects in the exposed subject.

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References

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نتیجه‌گیری:

품یصدی وابسته به تغییرات فیزیولوژیکی بر روی تنفس نیازمند بررسی و تحقیق بیشتری می‌باشد.

کلیدواژه‌ها: دیازپنون، سطح خونی وابسته، تناسق یوستیو، خروکش