Responses of serum calcium and inorganic phosphate levels as well as parathyroid gland and calcitonin producing C cells of Rattus norvegicus to Mipcin administration

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Abstract: Serum calcium (Ca) level of Rattus norvegicus ranged between 13.08±0.41-13.25± 0.39 mg/100 ml whereas serum inorganic phosphate (Pi) concentration varied between 4.21± 0.28 - 4.33 ± 0.26 mg/100 ml. Sublethal (0.50 LD₅₀ and 0.75 LD₅₀) administration of Mipcin induced a progressive dose-dependent decline in serum Ca level in the rat which was statistically significant at 7 and 14 days. Serum inorganic phosphate level of the treated rats did not exhibit significant fluctuation during the entire course of investigation. Parathyroid chief cells of the experimental rats exhibited degradation, vacuolation, loss of secretory (hormone) granules and lipid droplets, decreased chromatin in nuclei and damages in the endoplasmic reticulum as well as cristae of mitochondria at 14 days of the treatment. Not much of changes could be seen in the oxyphil cells of parathyroid as well as thyroid C cells of the Mipcin-treated rats.

Key words: Mipcin, Serum calcium, Serum inorganic phosphate, Parathyroid gland, Oxyphil cells, C cells, Rattus norvegicus

Introduction

Mipcin (2-isopropylphenyl-N-methylcarbamate), a carbamate insecticide developed by Mitsubishi Chemical Industries Limited, Japan is extensively used for the control of sucking pests of cotton, plant hoppers of rice and aphids of safflower (Murthy et al., 1990; Huang and Pang, 1992; Yang et al., 1995; Anon, 2005a). Effects of the spray of this insecticide on the farm labourers (mixers, loaders and sprayers) and livestock (calves, sheep, dogs and birds) have been evaluated recently by More et al. (2003a, b). So far nothing is known about the effects of Mipcin (C₁₁H₁₉O₂N) on the endocrine glands related to plasma calcium (Ca) and inorganic phosphate (Pi) metabolism of vertebrates (Dacke et al., 1996; Anon, 2005b). Since parathyroid hormone (PTH) and calcitonin (CT) play major role in plasma calcium (Ca) and inorganic phosphate (Pi) regulation of mammalian vertebrate (Pang and Schreibman, 1989; Wendelaar Bonga and Pang, 1991; Pandey, 1991, 1992; Aurbach et al., 1992; Dacke et al., 1996), an attempt has been made to record the responses of serum calcium (Ca) and inorganic phosphate (Pi) levels as well as parathyroid gland and C cells of Rattus norvegicus to sublethal Mipcin administration.

Materials and Methods

Healthy male rats, Rattus norvegicus (weighing 150-200 g) procured from the Bombay Municipal Corporation, Mumbai were acclimatized under the ambient laboratory conditions (temperature 28±2°C; photoperiod 14 L : 10 D) for 10 days. They were fed ad libitum on rat feed (Lipton, Bangalore) and clean water was provided for drinking. Sixty male rats were randomly selected and divided into 3 equal groups - two experimental and one control. Technical grade Mipcin (50 WP: Mitsubishi Chemical Industries Limited, Japan) was initially dissolved in small quantity of ethyl alcohol and diluted with physiological saline to prepare the two test doses: 0.50 LD₅₀ (182 mg/kg body weight) and 0.75 LD₅₀ (234 mg/kg body weight). Intramuscular (im) injections of both the doses were given daily to the experimental rats for 14 days whereas the control group animal received an equal volume (0.2 ml) of physiological saline. Blood samples were collected from post-caval vein of the rats under mild ether anesthesia at 24 hr, 7 and 14 days and centrifuged at 3,500 rpm to separate serum. Serum calcium (Ca) and inorganic phosphate (Pi) levels were estimated according to Trinder (1960) and Fiske and Subbarow (1925) methods, respectively. Values obtained for the control and experimental rats were evaluated for statistical significance using Students 't' test.

Both parathyroid and thyroid glands were surgically removed and fixed immediately in Bouin’s solution for light microscopic studies. After routine processing, sections were cut at 6 µm and stained with hematoxylin-eosin (H and E) and lead-hematoxylin (Solcia et al., 1969). For electron microscopic observations, the tissues were fixed in 3% glutaraldehyde maintained at 4°C. Thereafter, they were washed thoroughly with 0.1N cacodylate buffer to remove traces of glutaraldehyde and kept in 1% osmium tetroxide for 2 hr at 4°C. The tissues were
dehydrated through a series of alcohol, cleared in propylene oxide and transferred to the mixture of equal parts of propylene oxide and araldite solution for 1 hr to facilitate infiltration. They were kept overnight in araldite solution A and then in the araldite solution B for 1 hr at room temperature.

For preparation of tissue blocks, both the glands were embedded in plastic capsule KDB filled with araldite solution B. They were kept at 60°C for 48 hr for polymerization and hardening. The blocks were removed from the capsule and trimmed with a surgical blade under stereomicroscope. Semi-thin sections (1μm) were cut using ultramicrotome, spread on glass slides and fixed by gentle heating. The sections were stained with toluidine blue and examined under the light microscope. Ultra-thin sections (600-800 A²) were cut from the selected area with glass knife and mounted on 400 mesh copper grids. The tissues were double stained with 10% alcoholic uranyl acetate for 20 min and Reynold’s lead citrate for 10 minutes. Sections were scanned under Jeol-100 electron microscope.

Results and Discussion
Serum calcium (Ca) level of the control Rattus norvegicus ranged between 13.08±0.41-13.25±0.39 mg/100 ml whereas serum inorganic phosphate (Pi) value fluctuated between 4.21±0.28-4.33±0.26 mg/100 ml. Intramuscular (Im) administration of Mipcin induced a progressive dose-dependant decline in serum calcium (Ca) level in the rats which was statistically significant at 7 and 14 days. However, the hypocalcemia was more pronounced at 0.75 LD₅₀ dose of the pesticide as compared to 0.50 LD₅₀ (Table 1). Serum inorganic phosphate (Pi) level of the treated rats did not exhibit significant fluctuation during the entire course of investigation (Table 1).

Parathyroid gland of the control R. norvegicus consisted mainly of chief cells arranged in elongated and branching cords, separated by connective tissue stroma, capillaries and sinusoids (Fig. 1). Electron microscopic observations revealed that the chief cells were bound by unit membranes, clear desmosomes and terminal bars joining the plasma membranes. The nucleus was large, spherical or oval structure containing many small granules which were more concentrated towards the periphery. The cytoplasm contained prominent rough endoplasmic reticulum. The Golgi apparatus was composed of straight or curved stacks or membranes with small vesicles and granules. The mitochondria were distributed throughout the cytoplasm (Fig. 5). Only a few electron dense secretory granules could be seen in the cytoplasm. Besides these, lipid, glycogen and lysosomal bodies were also noticed in the chief cells. Mipcin treatment for 14 days elicited decrease in degranulation and vacuolation in parathyroid chief cells. Some pyknotic nuclei were also encountered in chief cells of the treated rats (Fig. 2). Further, the chief cells exhibited depletion of secretory (hormone) granules and lipid droplets in the rats injected with Mipcin. There was also decrease in chromatin content of the nuclei of chief cells; however, the degenerative changes (damages) were more prominent in endoplasmic reticulum and cristae of the mitochondria of the experimental rats which were more pronounced in the chief cells of the rats treated with 0.75 LD₅₀ than those given 0.50 LD₅₀ Mipcin (Fig. 6).

A few oxyphil cells were also encountered in the parathyroid gland of R. norvegicus. They were polygonal in shape, possessed smaller, irregular and denser nuclei than the chief cells. The abundant cytoplasmic area of the oxyphil cells was filled with numerous large mitochondria. The endoplasmic reticulum, Golgi apparatus and secretory granules were poorly developed in these cells (Fig. 7). Mipcin administration even for 14 days did not produce much ultrastructural changes in oxyphil cells of R. norvegicus (Fig. 8).

Calcitonin producing C cells of R. norvegicus were unevenly distributed in the thyroid follicular cells. They were larger in size with more transparent cytoplasm as compared to those of follicular cells (Fig. 3). Ultrastructurally, they possessed conspicuous endoplasmic reticulum, prominent Golgi apparatus, numerous mitochondria (both circular and elongated types) and dark electron dense secretory granules in their cytoplasm (Fig. 9). In few cells, desmosomes and terminal bars were also encountered. Mipcin administration for 14 days did not produce much, light microscopic (Fig. 4) as well as ultrastructural, changes in the thyroid C cells of R. norvegicus (Fig. 10).

Pesticides are being indiscriminately used in pest management as well as public health programmes all over the world (Anon, 2005a, b). Some of them are being marketed even before fully realizing their impact on non-target organisms and ecosystems (Brown, 1978; Hayes, 1982). There exists reports that addition of DDT in the feed of laying hen caused egg shell thinning, probably due to reproductive failure (Urist, 1976). Interestingly, administration of an organophosphate pesticide diazinon in eggs resulted in skeletal and spinal deformities in the chicks (Anon, 2005b). We have demonstrated, for the first time, a significant decline in serum

Table 1: Effect of intramuscular administration of Mipcin on serum calcium and inorganic phosphate levels (mg/100 ml) of Rattus norvegicus

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum calcium</th>
<th>Serum inorganic phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hr</td>
<td>7 days</td>
</tr>
<tr>
<td>Control</td>
<td>13.20±0.41</td>
<td>13.25±0.39</td>
</tr>
<tr>
<td>Mipcin (0.50LD₅₀)</td>
<td>12.94±0.47</td>
<td>12.14±0.37**</td>
</tr>
<tr>
<td>Mipcin (0.75LD₅₀)</td>
<td>12.80±0.38</td>
<td>11.47±0.34**</td>
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</tbody>
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Values are Mean±S. D. of 5 animals. Significant responses: *p < 0.05; **p < 0.001
Responses of serum calcium in Rattus to Mipcin administration

Fig. 1: Parathyroid gland of control R. norvegicus showing active chief cells. H and E. X 400

Fig. 2: Parathyroid gland of R. norvegicus at 14 days of Mipcin (0.75 LD_{50}) administration exhibiting degenerative changes in the chief cells. Mark the decrease in size of the chief cells and pyknotic nuclei (arrow). H and E. X 400

Fig. 3: Thyroid gland of the control R. norvegicus showing the distribution of C cells in follicular epithelium and interfollicular spaces (arrow). H and E. X 600

Fig. 4: Thyroid gland of R. norvegicus at 14 days of Mipcin (0.75 LD_{50}) administration exhibiting almost similar structure of C cells (arrow). H and E. X 800
Fig. 5: Ultrastructure of the control chief cell showing nucleus (N) with chromatin material towards periphery, Golgi complex (Gc), mitochondria (M) and endoplasmic reticulum, X 8,000

Fig. 6: Ultrastructure of the chief cell of *R. norvegicus* at 14 days of Mipsin (0.75 LD$_{50}$) administration exhibiting conspicuous vacuolation in the nucleus (N) and cytoplasm, X 31,000
Fig. 7: Oxyphil cell of the control rat showing chromatin-filled dense nucleus (N). X 3,000

Fig. 8: Oxyphil cell of the rat at 14 days of Mipcin (0.75 LD$_{50}$) administration showing almost normal structure with large number of mitochondria (M) and desmosomes (D). X 10,000
Fig. 9: Ultrastructure of C cell of the control *R. norvegicus* exhibiting conspicuous rough endoplasmic reticulum (Rer) and hormone granules (Hg). X 25,000

Fig. 10: Ultrastructure of the C cell of *R. norvegicus* at 14 days of Mipcin (0.75 LD<sub>50</sub>) administration depicting almost normal structure. Mark the presence of mitochondria of various shapes (M) and desmosomes (D). X 30,000
Changes in the parathyroid chief cells of the Mipcin-treated rats. Though decrease in serum Ca level has also been recorded in the catfish (Heteropneustes fossilis) exposed to aldrin (Singh et al., 1996), deltamethrin (Srivastav et al., 1997; Kumar et al., 1999) and cypermethrin (Mishra et al., 2001) but the physiological mechanism(s) of the manifestation is not yet known. Our observations demonstrate that the induced hypocalcemia in response to exogenous Mipcin administration in R. norvegicus is due to the degenerative changes in chief cells because the oxyphil as well as C cells remained almost unaffected to the treatment.

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Anon: Pesticides standardized in Punjab with dose(s), crop(s) and target pest(s). National Pak.Com Pakistan Agriculture Online. Agriculture Department Government of Punjab, Pakistan (2005a).


