Biochemical and Histopathological Effects of Carbendazim to Rat Male Reproduction

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SUMMARY

The purpose of this study was to determine the effects of carbendazim (methyl 2-benzimidazole carbamate) on the some hormone levels and on testis tissue. Carbendazim in the doses of 0, 150, 300, and 600 mg/kg/day were administered (by gavage) to male rats, daily for 15 weeks. At the end of the experiment, serum total testosterone and dihydrotestosterone (DHT) levels were analyzed, and testis tissues were taken for light and electron microscopic examinations.

A statistically significant decrease in total serum testosterone level in rats exposed to carbendazim (dose: 300 and 600 mg/kg/day), compared to the control, was observed. Also, dihydrotestosterone level was significantly decreased in all experimental groups. Histopathologically, carbendazim caused detrimental effects in testis tissues. These effects were vacuolization, disorganization and necrosis in germinial epithelium. In addition, multinucleated giant cells were observed in the lumen of the seminiferous tubules. These pathological findings were supported by electron microscopic examinations. These results suggest that carbendazim, in subchronic exposure, affects testis tissue and androgenic hormone levels.

Key words: Carbendazim; Rats; Reproductive Toxicity

INTRODUCTION

Carbendazim (methyl 2-benzimidazole carbamate) is a metabolite of the fungicide benomyl [methyl-1-(butylcarbamoyl)-2-benzimidazole carbamate] and is also used as a fungicide. The fungicidal properties of these compounds are the result of the binding of carbendazim to tubulins, an effect that disrupts microtubule formation and mitosis (Dustin, 1984; Burland, 1984). A number of studies have shown that carbendazim is able to damage the male reproductive system (Gray et al., 1990; Cummings et al., 1992). Significant decreases in fertility, along with declines in testi-
icular weight, epididymal sperm counts and was deferens sperm concentrations have been observed (Evenson et al., 1987; Gray et al., 1990). Also, subsequent studies have shown that carbendazim, in vivo or in vitro, perturbs microtubule dynamics during the completion of meiotic cell divisions (Smith et al., 1989). Can and Albertini (1997) have shown that carbendazim disrupts cell cycle progression in mouse oocytes by altering meiotic spindle microtubule stability and spindle pole integrity. Some authors concluded that carbendazim caused histopathological damage in thyroid, parathyroid and adrenal glands, and affected certain hormone levels in male rats (Barlas et al., 2002). Also, carbendazim administration changed some liver and kidney enzyme activities, and blood parameters of male rats (Selmanoglu et al., 2001). Cummings et al. (1990) have demonstrated that carbendazim is embryotoxic and also teratogenic when administered to rats in the dose of 400 mg/kg/day, during late pregnancy.

There are a lot of investigations about the short-term effects of carbendazim on the reproductive system. This study was conducted to examine the subchronic effects of carbendazim on testis tissue and total serum testosterone, and dihydrotestosterone levels in male rats.

MATERIAL AND METHODS

Chemicals

Carbendazim (methyl 2-benzimidazole carbamate), technical, purity 98% was obtained from Agro-San Drug Firm, Kirklareli, Turkey. Standard RIA hormone kits were purchased from Sangtec Diagnostica (Ankara, Turkey). All other chemicals used in these studies were of analytical grade.

Animals and Experimental Design

Male Wistar Swiss albino rats weighing 200-250 g at the beginning of the experiment, were used in this experiment. Animals were obtained from the Experimental Animal Production Center, Hacettepe University, Ankara, Turkey. During the experiment (15 weeks) animals were kept in standard stainless steel cages, under the same environmental conditions at a room temperature of 21±2°C, 12-h light-dark cycle, with food and water available ad libitum. A total of 50 male rats were randomly assigned into five equal groups. Three groups served as the experimental, and two were used as the standard and oil control groups. Experimental groups received (by gavage) carbendazim at doses of 150, 300 and 600 mg/kg body weight dissolved in corn oil (275 µl corn oil/rat/day), for a period of 15 weeks. The rats in the oil control group received
additional corn oil, which in equal amount as in experimental groups. Food and water consumption were measured daily, and animal body weight weekly.

**Biochemical Studies**

At the end of the experiment, the animals were killed by cervical dislocation, and blood was collected from heart. After centrifugation of the blood at 3200 rpm for 30 minutes, serum was separated and conserved at -20°C until hormone analyses. Serum total testosterone and dihydrotestosterone (DHT) were measured by radioimmunoassay technique using RIA kits.

**Histopathological Studies**

For histopathological examinations, testes were dissected and weighed (after excluding other tissues) in order to calculate the organ/body weight ratios for each animal. Then, the tissues were fixed in Bouin’s solution and processed in a series of graded ethanol, paraffin sections cut at 5-6 µm thickness, stained with Harris hematoxylin and eosin. From each testis five consecutive sections were examined with light microscopy for histopathological examination (Gurr, 1972).

For electron microscopic investigations, portions of testis were fixed by immersion in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH = 7.4). After a minimum of 2-4 h at 4°C, the tissues were washed three times for 20 min. in 0.1 M phosphate buffer. Secondary fixation was achieved by immersing the tissues specimens in 1% osmium tetroxide in phosphate buffer for 2-h at room temperature. Following three washes in buffer, specimens were dehydrated in graded ethanol and finally embedded in Araldite. Ultrathin sections 60-80 nm were cut on a Leica Ultracut R ultramicrotome. Prior to viewing, sections were counterstained with uranyl acetate followed by lead citrate. Sections were examined using a Leo 906 E electron microscope.

**Statistical Analyses**

The results were analyzed statistically using one way analysis of variance (ANOVA) followed by Dunnett’s test (SPSS package, version 7.0). Comparisons were made between the standard control and treatment groups (no difference between the standard and oil control groups was observed). A value of P <0.05 was taken as a statistically significant.

**RESULTS**

**Morphological Investigation**

Data obtained from organ weight and organ/body weight ratios of control and rats administered carben-
Table 1. Body weight, absolute and relative testis weight of control and male rats administered carbendazim for period of 15 weeks.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Kontrola</th>
<th>Dose of Carbendazim - Doza karbendazima</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(mg/kg/day)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>150</td>
</tr>
<tr>
<td>Body weight at start (g)</td>
<td>240.4±77.6</td>
<td>244.2±35.0</td>
</tr>
<tr>
<td>Telesna masa na početku (g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>315.0±75.8</td>
<td>339.3±56.9</td>
</tr>
<tr>
<td>Telesna masa na kraju (g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increase (%)</td>
<td>31.0</td>
<td>38.9</td>
</tr>
<tr>
<td>Povećanje (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute testis weight (g)</td>
<td>1.26±0.06</td>
<td>0.86±0.03</td>
</tr>
<tr>
<td>Apsolutna masa testisa (g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relative testis weight (x 10^-3)</td>
<td>4.76±1.1</td>
<td>2.59±5.4a</td>
</tr>
<tr>
<td>Relativna masa testisa (x 10^-3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values in table are means ± SD of 10 animals
Vrednosti u tabeli predstavljaju srednju vrednost ± SD od 10 životinja

aSignificantly different (P <0.05) from control (Dunnett’s test)

aStatistički značajna razlika (P <0.05) u poređenju sa kontrolom (Dunnett-test)

dazim are presented in Table 1. Growth was not markedly affected by carbendazim administration at any dose level. Relative testis weights were reduces by about % 40-45 of that of control at 150, 300 and 600 mg/kg/day doses groups (P<0.05).

Biochemical Investigations

The results of hormone analyses (all groups) are presented in Table 2. Serum dihydrotestosterone levels were significantly decreased (P<0.05), compared to the control, in all test groups, and total testosterone level was decreased in rats administered carbendazim in the doze of 300 and 600 mg/kg/day.

Histopathological Investigation

Histologically, the testis of control animals has a characteristic appearance showing all the seminiferous epithelium (Fig. 1). All stages of transformation of the seminiferous epithelium from spermatogonia to mature spermatooza are seen in the tubules. The effects of carbendazim on testicular histology are shown in Figs. 2-4. At dose of 150 mg/kg, slight disruption of the seminiferous tubular architecture was observed. At dose of 300 mg/kg histopathological
### Table 2. Serum hormone levels in control and male rats administered carbendazim for period of 15 weeks.

<table>
<thead>
<tr>
<th>Parameter - Parametar</th>
<th>Control Kontrola</th>
<th>Dose of Carbendazim - Doza karbendazima (mg/kg/day)</th>
<th>Dose of Carbendazim - Doza karbendazima (mg/kg/dan)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total testosterone (ng/ml)</td>
<td>1.94±0.5</td>
<td>1.92±0.5</td>
<td>1.48±0.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ukupni testosteron (ng/ml)</td>
<td>1.92±0.5</td>
<td>1.48±0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.45±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dihydrotestosterone (µg/ml)</td>
<td>0.31±0.1</td>
<td>0.152±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.11±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values in table are means ±SD of 10 animals
Vrednosti u tabeli predstavljaju srednju vrednost ± SD od 10 životinja

<sup>a</sup>Significantly different (P <0.05) from control (Dunnett's test)
<sup>a</sup>Statistički značajna razlika (P <0.05) u poređenju sa kontrolom (Dunnett-test)

... damages included a wide range of disorganization, vacuolization in germinal epithelium, and congestion. Also, the Sertoli cells appeared thoroughly vacuolated. The maximum histopathological damage was observed in animals treated with 600 mg/kg. In this group (600 mg/kg) many of seminiferous tubules had become atrophic and necrotic, and slo-

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**Fig. 1.** Structure (normal) of the seminiferous tubules in testis of the control animals (H&E x 450)

**Sl. 1.** Normalna struktura semenonsnih tubula testisa kontrolnih životinja (H&E x 450)
Fig. 2. Seminiferous tubular degeneration, vacuolization (arrowheads), congestion in testis interstitial tissue (arrows) of rats administered carbendazim (300 mg/kg) (H&E x 450)

Sl. 2. Degenerisane semenonosne tubule, vakuolizacija (vrhovi strelica), kongestije u intersicijalnom tkivu (strelice) testisa pacova koji su dobijali karbendazim u dozi od 300 mg/kg (H&E x 450)

Fig. 3. Seminiferous tubular degeneration, multinucleated giant cells (arrows), and sloughing (arrowheads) in testis of rats administered carbendazim (600 mg/kg) (H&E x 450)

Sl. 3. Degenerisane semenonosne tubule, multiijedarine čelije (strelice) i krastice (vrhovi strelica) u testisima pacova koji su dobijali karbendazim u dozi od 600 mg/kg (H&E x 450)
Fig. 4. Atrophic seminiferous tubules devoid of germinal cells in testis of rats administered carbendazim (600 mg/kg) (H&E x 180)

Sl. 4. Atrofiya semenonosnih tubula bez germinalnih čelija u testisima pacova koji su dobijali carbendazim u dozi od 600 mg/kg (H&E x 180)

Fig. 5. EM micrograph of rats administered carbendazim (300 mg/kg). Round and angled shaped nuclei of spermatozoons (†), microtubuli bundles (m) in the apical cytoplasm of the Sertoli cell (x 4646)

Sl. 5. Okrugla i uglasta jedra spermatozoida, snopovi (paketići) mikrotubula (m) u apikalnoj citoplazmi Sertoli čelija testisa pacova koji su dobijali carbendazim u dozi od 300 mg/kg (x 4646)
ughing of the germ cells with the formation of multinucleated giant cells was observed.

Light microscopic findings were supported by electron microscopic investigation. Necrosis and apoptosis were increased in seminifer epithelium according to increasing doses of the treatment. In low doses (150 and 300 mg/kg/day) oval and round nuclei shape spermatozoons were seen settling down to the deeper region of the seminifer epithelium. Acrosome formation was still occurred in the group of 150 mg/kg/day but it was insufficient in 300 mg/kg/day group. In both of this groups round and angled shaped spermatozoons were seen (Fig. 5). Disorganize tail formation was seen because of microtubuli degeneration. Irregular microtubuli bundles and central gathering microtubuli assembles were detected in all groups (Fig. 6). In 600 mg/kg/day group, accelerating rate of apoptosis in different germ cells was noticed. Sertoli cell’s nuclei was normal appearance but mitochondrial changes, increasing the number of primer and seconder lysosomes were seen. In their cytoplasm, there were also many changing size and electron dense precipitates. The basal membrane of seminifer epithelium became thick and big vacuoles were detected (Fig. 7). Multinucleated giant cells were seen in the seminifer tubuli (Fig. 8).

Fig. 6. EM micrograph of rats administered carbendazim (300 mg/kg) Degenerative mitochondria (m) and central gathering of microtubule (arrow) (x 27800)
Sl. 6. Degenerične mitohondrije (m) i centralno skupljene mikrotubule (strelica) u testisima pacova koji su dobijali karbendazim u dozi od 300 mg/kg (x 27800)
DISCUSSION

The effects of carbendazim on male rats testis was evaluated biochemically and histopathologically. Carbendazim produced significant alterations in serum testosterone and dihydrotestosterone (DHT) levels. Testosterone is the most important androgen. In the male, the interstitial cells (Leydig cells) of the testis produce testosterone. This hormone promotes the production of functional sperm, maintains the secretory glands of the male reproductive tract and stimulates growth. In many target tissues some of the testosterone is converted to dihydrotestosterone (DHT). A small amount of DHT diffuses back out of the cell and into the circulation, and DHT levels are usually about 10 percent of circulating testosterone level (Martini, 1995). Levels of testosterone found in this study were not similar to those previously reported for male rat (Rehnberg et al. 1989; Gray et al. 1990). These authors have concluded that serum testosterone levels were not different from controls, at 200 and 400 mg/kg carbendazim, although 200 and 400 mg/kg elevated androgen binding protein concentrations in serum and seminiferous tu-
bules. In the present study, serum testosterone and dihydrotestosterone levels decreased significantly.

Carbendazim may act indirectly to suppress the secretion of gonadotropin. Therefore, hormonal imbalance might occur due to impairment of either synthesis or secretion (Özmen and Akay, 1993). Goldman et al. (1989) reported that carbendazim acts on the hypothalamic-pituitary endocrine axis indirectly, through effects on the Sertoli cell. Also, ingestion of this fungicide did produce a functional alteration in the male reproductive system as assessed relative organ weights and some histopathological changes in testis. Relative testis weights was significantly decreased depending on the treatment doses. It has been reported that 1.0, 6.3 or 203 ppm benomyl (70 day feeding course) caused a significant decrease in relative testicular weights and a lowered male fertility index, but not in plasma testosterone, LH or FSH levels (Barnes et al., 1983).
In this study, the testis were swollen and contained degenerative and atrophic seminiferous tubules. This is attributable to the continued production of the seminiferous tubule fluid (Gotoh et al. 1999). Also, the sperm production in these testis was probably reduced. In the our study, the epithelium and/or the lumen contained multinucleate giant cells with several nuclei containing marginalized chromatins. The occurrence of multinucleate giant cells towards the lumen may suggest that during mitosis of the spermatogonia and meiosis of the spermatocytes, subsequent to division of the nucleus the cells fail to undergo cytokinesis. In addition, sloughing of the germ cells with the formation of multinucleated giant cells was observed. Sloughing is caused by the effects of the chemical on microtubules and intermediate filaments of the Sertioli cells (Hess and Nakai, 2000). A lot of authors demonstrated that carbendazim caused seminiferous tubular atrophy, total testicular atrophy and infertility (Carter and Laskey, 1982; Carter et al., 1987; Hess et al., 1991; Nakai et al., 1992). In addition to this findings, Nakai et al. (1997 & 1998) reported that carbendazim induced various morphological abnormalities in round and elongating spermatids. According to these results, it is possible that carbendazim inhibits acrosome development in the early phases of spermiogenesis. A single, high dose (100 mg/kg) of orally administered carbendazim prohibited supply of maternal from the Golgi apparatus to the acrosome and impairment of acrosome development.

As a conclusion, these results indicate that subchronic administration of carbendazim resulted in decrease in serum testosterone and dihydroteosterone hormone levels, and histopathological damage in the testis.

REFERENCES


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Biohemijski i histopatološki efekti Karbendazima na reprodukciju mužjaka pacova

REZIME

Svrha ovih ispitivanja bila je da se utvrdite efekti karbendazima (metil 2-benzimidazol karbamat) na nivo nekih hormona i na tkivo testisa. Karbendazim u dozama 0, 150, 300 i 600 mg/kg/dan je doziran oralno (sondom u želudac) mužjacima pacova, svakodnevno tokom 15 nedelja. Na kraju eksperimenta, analiziran je nivo ukupnog testosterona i nivo dihidrotestosterona (DHT) u serumu, a tkivo testisa je uzimano za ispitivanja svetlosnom i elektronskom mikroskopijom.

Značajno smanjenje nivoa ukupnog testosterona, u poređenju sa kontrolom, registrovano je kod pacova koji su dobijali karbendazim u dozama od 300 i 600 mg/kg/dan, dok je nivo dihidrotestosterona bio značajno smanjen kod svih eksperimentalnih grupa. Histopatološki, karbendazim je ispoljio štetne efekte na testise koji se ispoljavaju kao vakuolizacija, dezorganizacija i nekroza germinalnog epitela. Takođe, registrovane su uvećane multijedane ćelije u lumenu semenosnih tubula. Patološki nalazi su potvrđeni elektronskom mikroskopijom.

Ovi rezultati pokazuju da karbendazim, pri subhroničnoj izloženosti ispoljava štetne efekte na tkivo testisa i nivo androgenih hormona.

Ključne reči: Karbendazim; Pacov; Reproduktivna toksičnost