THE EFFECT OF TESTOSTERONE PROPIONATE SUPPLEMENT ON TESTICULAR TOXICITY WITH THIAMPHENICOL IN MALE SPRAGUE-DAWLEY RATS

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ABSTRACT — Testosterone propionate (TP) was supplemented to male rats for assessment of its ameliorating effect on testicular toxicity with thiamphenicol (TAP). A total of 20 male Sprague-Dawley rats were treated orally with TAP at 200 mg/kg/day for up to 4 weeks. In addition, 5 male rats were allotted to the control group receiving vehicle only. Ten of the 20 treated rats had a Silastic capsule® (containing about 80 mg of TP) implanted in the dorsal skin at Week 2 and assigned to the TAP-TP group, while the other 10 treated rats were in the TAP group. After Weeks 3 and 4, five of both treated groups were examined for weight and histology of the testis and accessory genital glands, and for staging analysis of the seminiferous tubules. The same parameters were also assessed in the control group after Week 4. Weights and morphology of the seminal vesicle and prostate recovered remarkably from the TAP toxicity after TP supplement. However, no ameliorating effects of TP were obtained for the testis in either weight, morphology, or staging analysis of the seminiferous tubules.

KEY WORDS: Thiamphenicol, Male rats, Testicular toxicity, Testosterone supplement

INTRODUCTION

Thiamphenicol (TAP), a class of chloramphenicol antibiotics, is mainly applied to control infections by Vibrio parahaemolyticus or Escherichia coli in medical and veterinary clinics and also used widely in fish breeding industries. Major concern of the side effects or toxicity of TAP has been focused on disorder of erythropoiesis among patients or experimental animals, as similar in the case of chloramphenicol (Keiser, 1974; Takamizawa, 1984). There has been increasing evidence showing its potential of toxicity on the male reproductive system in experimental animals (Della Bella et al., 1996; Marubini et al., 1996; Ando et al., 1997). In our previous study, male Sprague-Dawley rats were treated with TAP at 0, 100, or 200 mg/kg/day, po, for 4 weeks, and then allowed to recover for 13 weeks (Maita et al., 1999). Staging analyses on the seminiferous tubules in the testis revealed significant decreases in indices of all types of germ cells in both treated groups, although the testes in the 100 mg/kg/day group appeared normal in routine histology. Rats in the 200 mg/kg/day group disclosed considerable damage of the germ cells but still retained the substantial architecture and germ cell arrangement in the seminiferous tubules. However, after a 13-week recovery period, their testes revealed an almost complete loss of total germ cell layers, showing the so-called Sertoli only syndrome. A time-course study was carried out in male Sprague-Dawley rats receiving TAP at 200 mg/kg/day, po, for 4 weeks followed by a recovery period for 10 weeks (Maita et al., 2003). The first changes in
staging analysis were found in decreased indices of spermatogonia (probably type B spermatogonia) in stage V and preleptotene spermatocytes in stage VII at Week 2. An intact, or a fully recovered, seminiferous tubule was frequently seen neighboring another one showing “Sertoli only syndrome”. All previous evidence suggested that the primary target of TAP toxicity was Sertoli cells. However, that may not rule out a possible role of other factors including Leydig cells, because deficiency of TES supply following Leydig cell damage might compromise Sertoli cell function leading to significant changes of germ cells consequently. In the present study, the effects of TES supplement on germ cell damage were examined in male Sprague-Dawley rats which had a capsule loading testosterone propionate (TP) implanted in the dorsal skin from Week 2 during the treatment with TAP at 200 mg/kg/day, po, for 4 weeks.

MATERIALS AND METHODS

Chemical

Thiamphenicol (TAP) of technical grade with 99.4% purity was purchased from Sansho Co. Ltd. (Bunkyo-ku, Tokyo). TAP was suspended into 0.5% carbonate methyl cellulose (CMC) solution and administered to animals.

Animals and housing

The animals used in the present study were reared, treated, and killed in accordance with the provisions for animal welfare in The Institute of Environmental Toxicology which follow the Guidelines for Animal Experimentation issued by the Japanese Association for Laboratory Animal Science (1987). A total of 25 male Sprague-Dawley rats of SPF origin (Crj:CD) were obtained at 8 weeks of age from Charles River Japan Inc., and housed in stainless steel cages with a wire-mesh floor in an animal room with a controlled temperature at 24±3°C, humidity at 55±15%, and ventilation at 10 times or more an hour. The rats were allowed free access to pellet chow MF (Oriental Yeast Co. Ltd., Itabashi-ku, Tokyo) and local tap water during the study. After an acclimatization period for a week, 20 rats were treated with 200 mg/kg of TAP in a volume of 5 ml/kg, 7 days a week, for up to 4 weeks. As the dose of 200 mg/kg/day could produce surely toxic but not destructive damage to the testis, it was chosen for the present study. The other 5 rats received the same volume of CMC only and served as the control group. At Week 2, 10 of the treated rats were selected randomly and had implanted subcutaneously a Silastic capsule® (10 mm long, 4 mm inner diameter; Dow Corning Corporation Medical Products, Mich, USA) loading crystalline testosterone propionate (TP) in the dorsal skin (Lue et al., 2000; Takikawa and Wakabayashi, 1994) and were assigned to the TAP-TP group, while the other 10 treated rats were assigned to the TAP group. A Silastic capsule® was loaded at about 80 mg of TP. After Weeks 3, 5, both the TAP and TAP-TP groups were anesthetized, laparotomized, killed by excess bleeding though the vena cava, and necropsied. After Week 4, all rats in the treated and control groups were also necropsied.

Observation

Clinical signs and mobility were observed daily and body weight was measured weekly during the treatment. At necropsy after Weeks 3 and 4, the brain, testes, epididymides, seminal vesicles (together with coagulating glands), and prostate were removed, weighed, and fixed in neutral buffered 10% formalin, except the testes, for histological examinations. The testes were immersed in FSA fixative (37% formalin 5.0; 5% sucrose solution 15.0; acetic acid 0.8 in a volume ratio) as mentioned in our previous report (Maita et al., 1999). Evaluation of organ weights in the present study was carried out for the ratio of each organ weight to brain weight (relative organ weight to brain). The testes were subjected to staging analysis of the seminiferous tubules in stage VII and the results were compared with those of the control group examined after Week 4. As the seminiferous tubules in stage VII provide almost an entire set of a germ cell line, they were chosen as the representative stage of the germ cell cycle. In a stage VII seminiferous tubule, total number of each type of germ cell was counted and divided by number of Sertoli cells in order to obtain the germ cell index.

Statistical analysis

Body weights, organ weights, and staging analyses were assessed by Student’s t test (Gad and Weil, 1994). A 5% or 1% level of probability was used as the criterion of significance.

RESULTS

Body weight change and organ weights

Growth rate of animals receiving TAP at 200 mg/kg/day was much retardted and no ameliorative effect was obtained following TP supplement (Table 1). Even
under such retarded growth, as net weights of the brain were comparable in rats of both treated and control groups, analyses of organ weight between the treated and control groups were conducted on a ratio of each organ weight to brain weight. Weights of the epididymides were not affected by the treatment and remained almost consistent during the treatment. The seminal vesicles and prostate seemed to be most vulnerable in terms of reduction of weight among the organs examined. With TP supplement, the weights of these organs showed a quick recovery even 1 week after commencement of the supplement (Week 3) and became comparable to the control group after Week 4. Whereas weight changes of the testes seemed to be different from those of the accessory genital glands, their weights at Week 3 were still in the control range. However, after Week 4 (with 2-week supplement of TP), the testicular weights in both treated groups were comparable with each other, but significantly lowered as compared with those of the control group. TP supplement failed to ameliorate the adverse effect of TAP on the testicular weights.

**Morphology**

At necropsy after Weeks 3 and 4, the seminal vesicle and prostate in the TAP group appeared very dwarfish and shabby, about half in size compared with those of the control group, and had lost the particular character of the tissue including secreta amount and nature. On microscope examination, the glandular epithelium was much attenuated and the secreta had lost their unique eosinophilic tincture. With supplement of TAP for 1 week (TAP-TP group), these accessory glands seemed to regain their original character considerably in both gross and microscopic features, although their sizes were still small. After Week 4 (with 2-week supplement of TP) there were no significant differences in size and morphology of these organs between the TAP-TP and control groups. As to the testes, there were no obvious differences in size and morphology between the treated (TAP, TAP-TP) and control groups after Week 3 except occasional basal lamina thickening of the seminiferous tubules and edematous areas in the stroma in the treated groups. Staging analyses of the seminiferous tubules could be conducted readily as mentioned below. However, after Week 4, the testes from both TAP and TAP-TP groups looked small in size and lost their unique tissue solidity as correspondent to weight reduction. On the microscope, degeneration and apoptosis of germ cells, probably of spermatocytes and spermatids, were frequently seen in the seminiferous tubules together with retention of sperm and proteinaceous fluid, dissociation and desquamation of germ cells, and frequent presence of multinuclear giant cells. In the germ cell layer, there were frequently vacuolar spaces which were probably formed after defoliation of the giant cells. There was no ameliorative effect of TP supplement on histological changes of the tissue as shown in Photo. 1 (control), 2 (TAP), and 3 (TAP-TP). Although the tissue constitution of the testis was heavily damaged in the treated groups, it was not difficult to find seminiferous tubules which appeared intact in routine histological examination and enabled us to conduct staging analyses. No conspicuous changes were observed in morphology of either Sertoli cells or Leydig cells.

**Table 1.** Organ weights in male rats after Week 3 and 4 of treatment with TAP at 200 mg/kg/day with or without loading of TP from Week 2.

<table>
<thead>
<tr>
<th>Study phase</th>
<th>Treatment</th>
<th>Week 3</th>
<th>Week 3</th>
<th>Week 4</th>
<th>Week 4</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg/kg/day)</td>
<td>200</td>
<td>200-TP</td>
<td>200</td>
<td>200-TP</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>318</td>
<td>337</td>
<td>▼292</td>
<td>▼265</td>
<td>426</td>
<td></td>
</tr>
<tr>
<td>No. animals examined</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Epididymides</td>
<td>46.0</td>
<td>39.9</td>
<td>42.7</td>
<td>43.0</td>
<td>51.8</td>
<td></td>
</tr>
<tr>
<td>Seminal vesicle</td>
<td>▼51.8</td>
<td>▼86.9</td>
<td>▼51.5</td>
<td>103.5</td>
<td>98.0</td>
<td></td>
</tr>
<tr>
<td>Prostate</td>
<td>▼14.5</td>
<td>▼24.3</td>
<td>▼12.2</td>
<td>26.0</td>
<td>31.3</td>
<td></td>
</tr>
<tr>
<td>Testes</td>
<td>146.4</td>
<td>142.3</td>
<td>▼109.2</td>
<td>▼109.2</td>
<td>151.8</td>
<td></td>
</tr>
</tbody>
</table>

a: The value is expressed as a ratio of organ weight to brain weight obtained at each period of measurement and compared statistically with those of the control group examined after week 4.

▼ or ▼: Significantly different from the concurrent control at 5% or 1% level of probability (Student's t test).
Staging analyses on stage VII seminiferous tubules showed that indices of preleptotene spermatocyte were significantly decreased in both TAP and TAP-TP groups as compared with that of the control after Week 3 (Fig. 1). In the TAP group, reduced indices were also presented for pachytene spermatocytes and round spermatid at this period. No significant differences were indicated superficially for indices of pachytene spermatocytes and round spermatid in the TAP-TP groups as compared to those of the control group after Week 3. There were no apparent differences in progress of tissue damage between the TAP

Photo 1. Seminiferous tubules of a male in the control group showing normal arrangement and distribution of sperms and germ cells at stage VII. Hematoxylin and eosin staining. ×190.

Photo 2. Seminiferous tubules of a male treated with TAP at 200 mg/kg/day for 4 weeks. Disarrangement of germ cells and frequent presence of multinucleated giant cells were noted. Sertoli cells appear normal. Hematoxylin and eosin staining. ×190.
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Photo 3. Seminiferous tubules of a male implanted with a Silastic capsule® of testosterone propionate from Week 2 during TAP treatment for 4 weeks, showing no ameliorative effects on tissue damage. ×190.

Fig. 1. Staging analysis of the seminiferous tubules on stage VII after Week 3 or 4 of treatment. control (killed at Week 4 of treatment), TAP group, TAP-TP group. SPG, spermatogonium; plSPC, preleptotene spermatocyte; pSPC, pachytene spermatocyte; rSPT round spermatid. *, **; p<0.05, p<0.01 (Student’s t-test: Index of each germ cell at each week of treatment was compared with that of the control examined after Week 4 of treatment.)
and TAP-TP groups after Week 4 and indices of all stages of spermatocytes and spermatid in these groups were significantly reduced as compared to those of the control.

**DISCUSSION**

As in the previous results (Maita et al., 1999; Maita et al., 2003), body weights decreased significantly following treatment with TAP at 200 mg/kg/day in the present study, so it might be suspected that the decreased spermatogenesis observed was the consequence of the malnutrition induced. However, it has been well documented that spermatogenesis is unaffected even under severe reduction of body weight and both arrangement and distribution of germ cells were well retained in male rats which had restricted food supply reducing weight as much as 70% of the control (Chapin et al., 1982). It is most likely that the impaired spermatogenesis shown in the treated groups (TAP, TAP-TP) was due to TAP treatment.

In line with cases of overdoses with other testicular toxicants, marked weight reduction and regressive changes of morphology in the seminal vesicles and prostate were sensitive markers of the toxicity in male Sprague-Dawley rats following the treatment with TAP. However, these organs showed a quick recovery after commencement of TP supplement and became completely comparable to the control in their weight and morphology after a 2-week supplement. The results suggested firstly that TES production in the testis was affected by TAP treatment, although it was unclear whether the change had resulted from the direct effect on Leydig cells or an indirect one following suppression of spermatogenesis. Secondly, it might be likely that the receptor sensitivity to TES was retained intact in these organs during the treatment with TAP. When talking about hormones and their dependent organs, one should take into consideration the pituitary-testicular axis in hormone synthesis. The relevance of that hormonal control can be ruled out in the present study because numerous clinical trials have shown no adverse effects on the pituitary-testicular axis by either TAP or chloramphenicol treatment (Keiser, 1974). TP supplement failed to either ameliorate or delay the progress of testicular toxicity of TAP, including weight loss and morphological damage to the testis as well as decreased indices of germ cells in staging analysis of the seminiferous tubules. Although indices of pachytene spermatocyte and round spermatid appeared intact superficially after Week 3 in the TAP-TP group, it was uncertain if TP supplement could delay the onset of TAP toxicity on the germ cells, because there were no apparent differences in progress of tissue damage between the TAP and TAP-TP groups. The present results strongly suggested that the implication of hormone influence, especially testosterone, was very small, if any, in testicular toxicity of TAP. A time course observation of germ cell damage by TAP was carried out in our previous study (Maita et al., 2003) and revealed that decreased indices of the germ cells were noted first for type B spermatogonia in Stage V and proleptotene spermatocytes in Stage VII after 2 weeks of treatment at 200 mg/kg/day, and followed by changes of their progenies thereafter. Testicular toxicity by TP aggravated with time, and even after cessation of the treatment, the tissue damage seemed to worsen, resulting in “Sertoli only syndrome”-like feature of the total or parts of the seminiferous tubules. However, when we applied BrdU labeling on these “Sertoli only syndrome”-like seminiferous tubules, positive cells could occasionally be seen on the periphery of the tubules (Maita et al., 2003). As Sertoli cells have no potential to proliferate, just like nerve cells, those positive cells should be type A spermatogonia or stem cells which are considered to be resistant to chemical injury. It was probable that the proliferating potential could be retained even in those “Sertoli only syndrome”-like seminiferous tubules, but in fact spermatogenesis was not initiated due to something wrong. Type B spermatogonia and their progenies are all nursed by Sertoli cells, and the whole process of spermatogenesis including differentiation, development, and maturation of germ cells is controlled by Sertoli cells. Therefore, once Sertoli cells die, all germ cells are deemed to die with no chance to recover. Even if type A spermatogonia or stem cells are still alive in the seminiferous tubules, they will never be able to step up into a line of spermatogenesis. It is quite likely that the primary target of TAP testicular toxicity is Sertoli cells, as discussed in our previous reports (Maita et al., 1999, 2003). Future studies will be focused on the mechanism of Sertoli cell damage following TAP treatment.

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Testosterone supplement on testicular toxicity with thiamphenicol.

REFERENCES


