The Effect of Heparin on the Carbon Tetrachloride Induced Changes in Rat Testis

Elcin Hakan Terzi1, Aysel Kükner3, Serkan Öztürk2, Candan Özogul3, Ugur Üyetürk4

1Department of Histology and Embryology, Abant Izzet Baysal University Medical School, Bolu, Turkey
2Department of Pediatric Surgery, Abant Izzet Baysal University Medical School, Bolu, Turkey
3Department of Histology and Embryology, Gazi University Medical School, Ankara, Turkey
4Department of Urology, Abant Izzet Baysal University Medical School, Bolu, Turkey

Abstract

Objectives: To investigate the preventive effects of low molecular weight heparin on testis destruction created by low dose (0.25 ml/kg) carbon tetrachloride (CCl4) application.

Methods: Sixteen Spraque Dawley male rats were used. CCl4 induced testicular destruction was created. There were 4 rats in each group; Group I received 1 ml of intraperitoneal olive oil, Group received intraperitoneal 0.25ml/kg CCL4 in 1 ml of olive oil, Group III received intraperitoneal 0.25ml/kg CCL4 in 1 ml of olive oil plus subcutaneous 180 IU/kg low molecular weight heparin and Group IV received only subcutaneous 180 IU/kg low molecular weight heparin. At the end of 4 weeks of study period, rats were sacrificed and tissue samples were fixed in glutaraldehyde solution.

Results: There was no statistically significant difference between the groups in terms of testis weight (p>0.05). Light microscopy showed vacuolization in the basal portion of the seminiferous tubules in Group II. Intercellular dehiscence between the spermatogenetic cells was significant in Group II compared to control group at the level of fine structure. Intercellular dehiscence was not significant in Group III compared to Group II. The seminiferous tubules structures in Group IV were found comparable with the control group. Low dose of CCl4 caused germ cell loss, inhibition of mitosis, and structural deterioration of the Sertoli cells in seminiferous tubules of rat testes.

Conclusions: Low molecular weight heparin was found to be effective preventing CCl4 induced testis destruction.

Keywords: Testes, Carbon tetrachloride, Enoxaparin sodium.

Introduction

Carbon tetrachloride (CCl4) is a colorless, liquid organic compound which has well-known hepatotoxic and nephrotoxic activities (1). It is still being used in the fumigation of grains, as an insecticide in agriculture, in dry cleaning and in filling fire extinguishers. Therefore, certain industrial workers are at risk of CCl4 related toxicity particularly liver injury during their life. Metabolic activation of CCl4 by cytochrome P450 system to the free radicals is reported to enhance oxidation of lipids, proteins and resulting in widespread membrane damage (2,3). Hepatic insufficiency eventually lead to a devastating clinical condition that often results in multiorgan failure (4,5).

Oxidative stress is known to show detrimental effects on the testis function via the induction of peroxidative damage to the plasma membrane (6). In consonance with the present study, Fadhel and Arman (7), Manjrekar et al (8) and Khan and Ahmed (9) showed that CCl4 intoxication induced enhancement in MDA level and depletion in GSH in the testes of the treated rats. Horn et al. induced alterations in the spermatogenic cycle and degeneration in seminiferous tubules using CCl4 in rats (1). Soliman et al. (10) evaluated the protective and curative effects of the 15 KD protein isolated from the seeds of Peganum harmala L. against carbon tetrachloride (CCl4) induced oxidative stress in rat testes. The authors reported that the treatment of rats either pre or post CCl4 intoxication successfully alleviated the oxidative stress in testes.

Figure 1. A. Dehiscence between the germ cells and extensive hemorrhage and congestion in the intertubular space were observed in Group II (H&E, bar 100 µm). B. Less pronounced germ cell dehiscence and no intertubular hemorrhage were observed in Group III (H&E, bar 100 µm).
Low-molecular-weight heparins (LMWH) are fragments of commercial-grade heparin produced by either chemical or enzymatic depolymerization and prophylactic agents in situations that lead to a high risk of thrombosis (11). Enoxaparin binds to and accelerates the activity of antithrombin III and its anticoagulant effect is directly correlated to its ability to inhibit factor Xa. Besides the antithrombotic effects, LMWHs are also have antiinflammatory, antiproliferative properties (12). Günther et al. reported the suppression of fibroproliferative response of rabbit lungs to bleomycin challenge with aerosolized heparin administration (13).

In this study, we have attempted to evaluate the protective effects of low molecular weight heparin at the cellular in CCl4 induced chronic testis toxicity in rats.

**Experimental procedure**

A total of 16 animals were divided into 4 groups with 4 rats in each groups:
- Group I (control group) received 1 ml intraperitoneal olive oil every other day,
- Group II received intraperitoneal 0.25 ml/kg of CCL4 in 1 ml olive oil every other day,
- Group III received intraperitoneal 0.25 ml/kg of CCL4 in 1 ml olive oil every other day and subcutaneously 180 IU/kg low molecular weight heparin (Enoxaparin sodium) daily, and
- Group IV received only subcutaneously 180 IU/kg enoxaparin sodium daily.

At the end of 4 weeks, 24 h after the last injection animals were anesthetized with ketamine/xylazine (90/10 mg/kg) and sacrificed by decapitation. After removal and weighing the testes were placed in 10% formalin and 2.5% glutaraldehyde for histological processing.

**Histologic examination**

Testis samples were fixed in 10% formalin, routinely processed and embedded in paraffin wax. Sections of 4-5 micrometer thickness were cut and stained with haematoxylin and eosin (HE).

Testis tissues were fixed in 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer, tissues were postfixed with 2% osmium tetroxide in sodium phosphate buffer. Dehydration was accomplished by gradual ethanol and propilen oxide series and tissues were embedded in epoxy resin. Sections of 1μm thickness were stained with Toluidine blue and 800 nm thicknesses were stained with lead citrate-uranil acetate. Thin sections were investigated under Zeiss Electron Microscopy and images were captured.
Testis weights per 100 gr of body weight were measured in all subjects. All the data presented as mean ± standard deviation. Kruskal Wallis test was used to compare the testis weight among the groups. The significance (P value) was set at the nominal level of 0.05.

Results

Mean (±standard deviation) testis weight per 100 gr body weight of the control group (Group I) was 0.79 ± 0.09 gr, Group II was 0.83 ± 0.03 gr, Group III was 0.86 ± 0.18 gr and Group IV was 0.90 ± 0.07 gr. There was no statistically significant difference between the groups in terms of testis weight (p>0.05).

Light microscopy showed vacuolization in some areas of the basal portion of the seminiferous tubules in Group II (Figure 1a). Vacuolar changes in other groups were not significant. The Sertoli cells were differentiated in all groups with its’ characteristic nucleolus structure. Less pronounced germ cell dehiscence and no intertubular hemorrhage were observed in Group III compared to Group II (Figure 1b).

At the level of fine structure, control group showed primary spermatocytes and early spermatids in seminiferous tubules. Sertoli cells and zolula occludens of blood-testis barrier were identified significantly (Figure 2a). While some of the spermatids were in the phase of acrosome cap formation, remaining spermatids showed heterochromatic nucleus and mature acrosome cap formation (Figure 2b). Intercellular dehiscence between the spermatogenetic cells was significant in Group II compared to control group. Vacuolization in the basal portion of the seminiferous tubules was also observed. Spermatogonium were located in basal compartment and primary spermatocytes and early spermatids were located in upper portion (Figure 3a). Zonula occludens between the Sertoli cells were usually intact (Figure 3b). Intercellular dehiscence was not significant in Group III compared to Group II (Figure 4a). In addition to this, spermatogenetic cells maintained the normal morphologic appearance (Figure 4b). The seminiferous tubules structures in Group IV were found comparable with the control group (Figure 5).

Administration of low dose of CCl4 caused germ cell loss, inhibition of mitosis, and structural deterioration of the Sertoli cells in seminiferous tubules of rat testes. The most pronounced changes were vacuolization and intercellular dehiscence. These changes were rarely observed in seminiferous tubules of rats in Group III.

Discussion

It is now well known that the target of free oxygen radicals related acute or chronic toxicity resulting from CCl4 is not only liver but also other tissues including heart, testis, lung, kidney, brain and blood (14). The present study demonstrated the protective role of enoxaparin sodium on CCl4 induced testis destruction at the level of fine structure.

Kalla and Bansal observed serious spermatogenetic cycle destruction including loss of germinal epithelium, empty germ cells and shrinkage of tubule structures after 20th days of initiation of CCl4 in rats (15). Besides this Horn et al. reported only regional changes in seminiferous tubule epithelium rather than extensive

Figure 4. (Group III). A. Intercellular dehiscence was not significant in Group III compared to Group II. B. Spermatogenetic cells maintained the normal morphologic appearance. (lead citrate/uranyl acetate staining).

Figure 5. (Group IV). The seminiferous tubules structures in Group IV were found comparable with the control group. Intercellular dehiscence was seen slightly in basal portions (lead citrate/uranyl acetate staining). Ps: Primary spermatocyte, Sc: Sertoli cell, S: Spermatogonium.
destruction in CCl₄ induced hepatic cirrhosis (1). In the present study, we detected vacuolization and intercellular dehiscence on rat seminiferous tubules after low dose exposure of CCl₄. Spermatogenetic and Sertoli cells prevented their normal structure and breakdown of tight junction complexes between Sertoli cells did not observed. There was no extensive degeneration on seminiferous tubules. We used a dose of 0.25 ml/kg of CCl₄ for chronic low dose testis toxicity model because Fadhel et al. did not observed significant lipid peroxidation difference among the three different doses of CCl₄ (7).

Abe et al. demonstrated the preventive role of deltaparin which is one of the LMWHs in CCl₄ induced chronic hepatic fibrosis model (16). Similarly, Abdel-Salam O.M et al. reported successful results using LMWH in hepatic injury and fibrosis model created by bile canal ligation (17). We documented in the present study the role of enoxaparine sodium in testicle injury induced by chronic CCl₄ administration. The possible preventive mechanism of enoxaparin may be due to less lipid peroxidation or secondary to its hepatoprotective effects.

Conclusion

In conclusion, low-molecular-weight heparin treatment ameliorated the cellular abnormalities occurring in the chronic low dose CCl₄ induced testis toxicity. Further studies are warranted to explain the protective role of low-molecular-weight heparin on biochemical changes and oxidative stress.

References