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L-Ascorbic acid partially protects two cycles of cisplatin chemotherapy-induced testis damage and oligo-astheno-teratospermia in a mouse model ☆

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Abstract

Cisplatin (*cis*-diaminedichloroplatinum-II) is a widely used antineoplastic agent in the treatment of a variety of cancers. The aim of the present study was to investigate the testicular toxicity of cisplatin in mice at human therapeutic dose-levels, and to investigate any protective effects of concomitantly administered L-ascorbic acid (i.p.; 10 mg/kg). Adult male BALB/C mice (13–15-week-old) were treated (i.p.) with two cycles of 5 days each of cisplatin with 17 days of recovery period between cycles, as follows: Group I (G-I) – water ($N = 10$); Group II (G-II) – L-ascorbic acid ($N = 6$); Group III (G-III) – 1 mg/kg ($N = 6$); Group IV (G-IV) – 1 mg/kg + L-ascorbic acid ($N = 6$); Group V (G-V) – 2.5 mg/kg ($N = 6$); and Group VI (G-VI) – 2.5 mg/kg + L-ascorbic acid ($N = 8$). All animals were sacrificed on third day after the last treatment. The testis weight was decreased in a dose-dependent pattern (G-III – 44% and G-V – 54% against G-I), but L-ascorbic acid (10 mg/kg) recovered the lost weight in G-VI up to 32% against G-V ($p < 0.05$). Seminiferous tubular pathology was indicated by vacuoles, epithelial gaps, epithelial sloughing, delayed spermiation, malorientation of spermatids, germ cell degeneration, phagocytosis of spermatids, multinucleated germ cell formation and atrophy. Structurally abnormal tubules (G-III – 33%; G-V – 100%) were induced, and protective effects were seen in G-IV (77%) and G-VI (25%; $p < 0.05$). The tubular diameter was decreased in G-III–VI, but recovery was seen only in G-IV. The epithelial height was decreased in G-III, G-V and G-VI and the recovery was seen only in G-VI. The sperm count was decreased up to 53% and 71% against control in G-III and G-V, respectively, and recovery up to 47% and 61% was observed in G-IV and G-VI, respectively. The sperm motility was decreased up to 56% and 63% against control in G-III and G-V, respectively, and recovery was only marginal in G-IV and G-VI ($p > 0.05$). Total sperm abnormalities were increased in G-III–V (274%, 156% and 232%, respectively, $p < 0.05$) and L-ascorbic acid protected the effect in G-VI up to 156% ($p < 0.05$). In conclusion, at human therapeutic dose-levels, cisplatin induces testicular damage and spermato-toxicity. L-Ascorbic acid only partially nullifies the gonadotoxic effects of cisplatin.

Keywords: Gonadotoxicity; Cancer chemotherapy; Testicular germ cell tumors; Antioxidants; Male infertility; Sperm morphology

Introduction

Testicular germ cell tumors are the common types of cancers affecting adult men, which could be effectively eliminated by a combination of radiotherapy and cisplatin (*cis*-diaminedichloroplatinum-II)-based chemotherapy

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resulting in a cure rate of about 95% (Mead and Stenning, 1997; Sawhney et al., 2005; Bieber et al., 2006). Cisplatin is used against a variety of neoplasms of head and neck, testis, ovary, urinary bladder, lungs and other organs. The testicular germ cell tumors (TGCTs) show least-acquired resistance to cisplatin although the reasons for such a unique effectiveness are not clear (Duale et al., 2007). Since patients suffering from TGCTs are usually of young age, and that in significant number of them the disease could be cured, the evaluation of reproductive function is extremely important since they look forward for a normal life in future (Bieber et al., 2006). One of the problems associated with cisplatin-based chemotherapy (bleomycin, etoposide and cisplatin; BEP) is the seminiferous epithelial damage and probably Leydig cell damage leading to delayed recovery of fertility or irreversible infertility (Howell and Shalet, 2001).

Cisplatin reacts with nucleophilic sites on DNA forming mono-adducts as well as inter- and intra-strand cross links (Wozniak and Blasiak, 2002). These properties of cisplatin inhibit cellular processes such as replication, transcription, translation and DNA repair (Wozniak and Blasiak, 2002; Sawhney et al., 2005). Despite its significant anticancer properties, the clinical use of cisplatin is often limited owing to its dose-limiting adverse effects on kidney, nervous system, liver and reproductive organs (Iraz et al., 2006). The mechanism of organ toxicity seems at least partly, if not absolutely, to be mediated via a pathway involving increased reactive oxygen and nitrogen species and decreased antioxidants (Naziroglu et al., 2004). Exposure to cisplatin in humans results in impairment of testicular functions, sometimes leading to permanent infertility, especially when a cumulative dose exceeds 600 mg/m^2 body surface area (Pont and Albrecht, 1997; Petersen and Hansen, 1999; Howell and Shalet, 2005). Cisplatin-induced oligospermia and azoospermia are due to impaired spermatogenesis following apoptotic germ cell death in the testis (Seaman et al., 2003). Exposure to 1, 2, or 4 cycles of cisplatin at dose-levels of 1, 2.5 or 5 mg/kg resulted in disruption of the testis structure mainly due to germ cell apoptosis, but also assisted by the Sertoli cell damage (Sawhney et al., 2005). In addition, cisplatin-based chemotherapy induces the formation of diploidy and disomy of chromosomes 16, 18, *X* and *Y*, hence rendering a chance to produce a genetically imbalanced offspring (De Mas et al., 2001). There are a few studies on effects of cisplatin on testicular function in animals; however, the major problem with them is the use of a very high single dose of the drug and arbitrary sample time selected to observe the effects (Ateşşahin et al., 2006a; Türk et al., 2008). Further, the single dose exposure and its subsequent effects on the testis are difficult to comprehend and compare to the effects of therapeutic dose-levels on the testis. Moreover, the effects of acute exposure, if any, are not indicative of doses of cisplatin and their effects, which are clinically relevant (Sawhney et al., 2005). Further, there are no studies evaluating the preventive effects of an antioxidant L-ascorbic acid on therapeutic dose-levels of cisplatin-induced testis damage. Invention of agents that protect the chemotherapy-induced gonadotoxicity is paramount, since it is not even possible to cryopreserve the semen from the patients owing to the cancer-induced alterations of semen parameters before the onset of chemotherapy (Kollmannsberger et al., 1999; Howell and Shalet, 2005), thus making it necessary to restore the quality of testicular function after the cessation of treatment.

L-Ascorbic acid is a scavenger antioxidant, which is known to improve the semen parameters in men (Eskenazi et al., 2005) and the level of this antioxidant is usually low in oligo-zoospermic and astheno-zoospermic patients (Ahmad et al., 2008). It is a major electron donor due to which reactive oxygen species are neutralized (Agarwal et al., 2005). L-Ascorbic acid that is present in the semen prevents the DNA damage; therefore effectively the low seminal level of this antioxidant is always associated with a higher incidence of DNA damage (Song et al., 2006), and an increased incidence of abnormal sperms (Thiele et al., 1995). Several toxic insults to the testis from several chemicals and social habits such as smoking, adversely affect the semen parameters by reducing the seminal ascorbic acid level (Narayana et al., 2005; Mostafa et al., 2006). The present study was designed to investigate the structural effects on the testis of two cycles of human therapeutic dose-levels of cisplatin and possible preventive effects of L-ascorbic acid in a mouse model.

Materials and methods

Animals

Adult male BALB/C mice (13–15-week-old) were acclimatized for a week before the onset of experiments. The animal room temperature was 25 °C and humidity was 50% with a 12:12 h light and dark cycle. Animals were given standard laboratory chow and water *ad libitum*. All animals were housed in plastic cages with saw dust bedding (5–6 mice/cage) with change of bedding every alternate day. All experimental procedures were conducted in accordance with the guidelines of Animal Ethics Committee of the Kuwait University.

Cisplatin treatment

Animals were segregated into 6 groups after a week long acclimatization period. The treatment schedule of