Role of Vitamin C and E on Sperm Abnormality and Sperm Count in Cadmium Treated Swiss Mice

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Summary In spite of the principal applications of Cadmium (Cd) in a variety of consumer and industrial based materials, the metal, during the recent past, has been branded as a potent environmental pollutant causing serious health hazards to almost all the organisms of the biosphere including human beings. The extensive mutagenic and carcinogenic spectrum of the metal, has warranted the humanity to restrict the undesired and abuse of the metal so as to save the planet from its dangerous clutches. The main objective of the present study is to demonstrate the effects of Cd on male germinal cells, the progenitor of sperm. The study reveals the generation of significantly higher quantities of reactive oxygen species (ROS) in Cd-treated Swiss mice testes which have detrimentally affected the germ cells consequently posing significant decline in sperm count compared to the untreated controls. Furthermore, the ROS generated through Cd catalysis have affected developing germ cells so as to produce varieties of morphologically abnormal sperm, the frequency of which increased significantly in Cd-treated mice than the controls. However, supplementation of vitamin C and E to Cd-treated mice, drastically reduced the generation of ROS, thereby significantly escalating sperm count and declining the frequency of abnormal sperm population. The study emphasises the possible role of the ROS in inducing sperm abnormality by way of altering specific gene loci in germ cell chromosomes. The probable role of the aforementioned vitamins in relieving the ROS-related injury to the germ cells has been discussed.

Key words Cadmium chloride, Lipid peroxidation, Vitamin C and E, Sperm abnormality and sperm count, Testis, Swiss mice.

Cadmium is an ubiquitous pollutant of the environment. Due to its extensive use and long biological half-life, the potential health hazards of Cd, on mankind have attracted much attention over the years. It is now, generally acknowledged that Cd exposure causes serious dysfunctions in renal, skeletal, vascular and respiratory tissues in humans (IARC Monographs 1992). Besides, Cd can potentially induce both mitotic and meiotic mutations in chromosome complements in a variety of organisms including human beings (Bui *et al.* 1975). However, reports on the effect of Cd compounds on male germinal cells particularly with regards to sperm count pattern and in the bearing of abnormal sperm population, seem to be very inconclusive and fragmentary. Fundamentally, altered sperm morphology in animals has long been known as a consequence of action of various physical and chemical agents on spermatogonial cells. Moreover, the phenotype of the sperm is defined in large measures by both autosomal and sex-linked genes, which are in exposure to mutagenic physical and chemical agents, change substantially in structure representing the abnormal sperm population. The sperm abnormality, therefore, provides comparatively a new approach to the assessment of damage occurring to germ cells and to screen out the mutagens which potentially bring about a stable transformation in the sperm genotype and morphology (Hollarden and Frederick 1978).

From the above point of view, the role of Cd compounds inducing sperm abnormality in mammals is noteworthy. Although, the sperm morphology in mice treated with cadmium chloride (CdCl₂) have been studied (Bruce and Heddle 1979, Mukherjee *et al.* 1988, Han *et al.* 1992); its re-

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lation to Cd-induced free radical actions on male germinal cells to produce abnormal sperm population have not yet been elaborately reported. Again, free radicals in CdCl₂ treated mice have allegedly damage most of the germ cells, thereby culminating a significant decline in total sperm count. In the present study, therefore, efforts have been taken to rectify the defective sperm population and to elevate the sperm count almost to the control level in CdCl₂ treated mice, by way of Vitamin C (VitC) and Vitamin E (VitE) supplementation, which are believed to play a key role in scavenging the free radicals and are able to protect the male germ cells from oxidative stress.

Materials and methods

Male albino mice of Swiss strain procured from a live animal supply farm at Kalkata, India, acclimated to laboratory conditions and fed with balanced diet and water *ad libitum*. From the reared stock, healthy mice of 10 to 12 weeks old and about 15 to 20 g body weight, were selected for study and divided into 4 groups, each consisting of 6 animals. The control group of mice were intraperitoneally injected with a dose of distilled water. Out of 3 experimental groups, one group of mice were injected with CdCl₂ (1 mg/kg body weight) while the second experimental group of mice were injected with CdCl₂ plus VitC (10 mg/kg body weight) and the third experimental group of mice were injected with CdCl₂ plus VitE (100 mg/kg body weight) through the same route and caged separately in the laboratory. Sacrifice of animals were done after 35 days of injection and sperm samples were collected from the *vas deferens*, and appropriately processed for the study of sperm counting and sperm abnormality tests as recommended earlier (Hollarden and Fredrick 1978). Sperm suspension was prepared in phosphate buffer saline and centrifuged. Small drops of suspension were taken on clear greaese free slides and smeared gently and dried overnight. Smeared slides were stained with 10% Giemsa (BDH) for 1 h and observed under microscope. Morphologically abnormal sperm were recorded as per Wyrobek and Bruce (1975). Percentage of sperm abnormality was calculated by scanning abnormal sperm. Sperm counting was done in haemocytometer using the sperm suspension.

For the study of free radical generation, lipid peroxidation potential (LPP) of testes was calculated following thio barbituric acid (TBA) test of Stroev and Makarova (1989) with minimum modification. Amount of thio barbituric acid-reactive substances (TBA-RS) in $\mu\text{mol/g}$ of wet tissue pro-

Fig. 1. Effect of VitC (10 mg/kg b.w, ip), VitE (100 mg/kg b.w, ip) on lipid peroxidation potential on the basis of TBA-RS in CdCl₂ treated mice (1 mg/kg b.w, ip).

duced was equivalent to the amount of free radicals generated.

Observation

Results of the present study emphasises a sharp and significant rise in TBA-RS content in the testicular tissue of CdCl_2 treated mice compared to vehicle control injected group. However, VitC or VitE supplementation to CdCl_2 group of mice, although, have substantially declined, the content of TBA-RS in the testicular tissue compared to CdCl_2 treated mice, but the parameter did not come down to the control level (Fig. 1).

Sperm counting pattern in CdCl_2 treated mice declined significantly in all the weeks of post treatment compared to control mice. Significant increase in sperm count has been observed in CdCl_2 treated mice supplemented with VitC and VitE (Fig. 2). VitC injected CdCl_2 treated mice, however, encountered with an increased sperm count value compared to the VitE supplemented

Fig. 2. Effect of VitC (10 mg/kg b.w, ip) and VitE (100 mg/kg b.w, ip) on sperm count ($\times 10^6$) in CdCl_2 (1 mg/kg b.w, ip) treated mice.

Fig. 3. Effect of VitC (10 mg/kg b.w, ip) and VitE (100 mg/kg b.w, ip) on sperm abnormality (in %) in CdCl_2 (1 mg/kg b.w, ip) treated mice.

mice (Fig. 2).

Different types of morphologically deformed sperm have been presented (Fig. 4a–l). Occurrence of abnormal sperm population in the control mice were significantly less than that of CdCl_2 treated mice. In contrast, CdCl_2 injected mice supplemented with VitC and VitE encountered with significantly decreased percentages of sperm abnormality compared to Cd treated mice (Fig. 3). Furthermore, CdCl_2 treated mice supplemented with VitE seemingly exhibited higher percentage of sperm abnormality compared to VitC supplemented mice (Fig. 3).

Discussion

Cadmium, like all other heavy and transitional metals, is known to damage intracellular biomolecules and membrane phospholipids through oxidative stress in laboratory animals (Fariss 1991, Manca *et al.* 1991, Koizumi and Li 1992, Sarkar *et al.* 1995). Significantly increased lipid peroxidation potential (LPP) in the Cd treated Swiss mice testes compared to the vehicle controls, in all the weeks of post treatment (5th and 8th) in the present context, however, finds enough support from the previous studies.

Significant reduction in sperm count level in mice following CdCl_2 treatment, in the present study, emphasizes the free radical mediated injury occurring to the spermatocytes at various stages of development in general and to the stem cells in particular, which eventually are transformed into mature sperm after many rounds of mitotic and meiotic divisions. Most of the spermatogonial cells, on the contrary, might have been detrimentally eliminated being damaged by toxic free radicals, thereby, posing a significant fall in the sperm count. The present supposition, however, is in agreement with the findings of the previous workers (Bleyle and Lewerenz 1980, Saygi *et al.* 1991, Miller and Alder 1992, Selypes *et al.* 1992, Bench *et al.* 1999).

Increased incidence of abnormal sperm, in the present context, is possibly a result of free radical mediated gene alteration in developing germ cells due to cadmium toxicity. It is already established that sperm shape abnormality is genetically controlled by various autosomal and Y linked genes (Bennett 1975, Krazanowska 1976). On the contrary, gene segments in chromosomes are believed to be altered and/or damaged being enacted by different types of reactive oxygen species (ROS) or free radicals generated through metal catalysed reactions (Ochi *et al.* 1987, Koizumi *et al.* 1992, Kawanishi *et al.* 1994, Reid *et al.* 1994). Hence, it may be reasonable to conceive that Cd catalysed free radicals possibly have become instrumental in mutating specific gene loci controlling sperm shape morphology, thereby, altering the sperm shape. Additionally, free radicals ensuing

Fig. 4. a) Normal sperm, b) and c) sperm head without hook, d–g) sperm with amorphous head, h) sperm with lance shaped head, i) sperm with double head, j) sperm with mid piece separated, k) sperm with folded midpiece, l) sperm with double tail. giemsa staining. $\times 450$.

from Cd treatment possibly have evoked genotoxic activity through transactivation of zinc finger proteins which supposedly have played a crucial role in altering specific gene loci responsible for the maintenance of appropriate sperm shape (Herr 1991, Sunderman 1998).

The present study also demonstrates the individual efficacy of the vitamins in neutralising oxidative stress-related germ cell injury in Cd treated mice. The Figs. 1–3 clearly emphasise that the vitamins are not fully potential to bring back the parameters to the control level. Secondly, the study also indicates higher potentiality of VitC in minimising testicular LPP and thereby increasing the sperm count level and reducing the percentage of morphologically abnormal sperm than VitE. Evidences suggest that both the vitamins are known to reduce free radical mediated genotoxicity in test animals (Colin *et al.* 1991, Ross and Moldeus 1991, Hoda and Sinha 1993). Over and above, both VitC and VitE have been tested in declining sperm abnormality in heavy metal induced rats (Hsu *et al.* 1998). A cursory survey of the available literature on functional behaviour of the aforementioned vitamins demonstrate that VitC, as an antioxidant, is able to directly scavenge the superoxide, hydroxyl and various lipid hydroperoxides while VitE is a potent chain terminator of lipid peroxidation process (Heffner and Repine 1989). In addition, VitC is believed to interact synergistically with VitE to enhance the antioxidative properties of the latter (Packer *et al.* 1979, Bascetta *et al.* 1983, Knekt 1994, Tappel 1997). Furthermore, VitC is crucial in recycling and regenerating VitE when they are in concert. The present experiment, however, has not been designed to supplement both the vitamins simultaneously to a group of Cd treated mice; therefore, the impact and advantage of the synergistic action of the vitamins, in the present set up, could not be fully experienced. Hence, further studies in this regard, may explain the utility and functioning of the vitamins to combat the impaired spermatogenesis process in Cd-intoxicated organisms.

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