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Full Length Research Paper

Prevention of cadmium-induced alteration in rat testes and prostate lipid patterns by α-tocopherol

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Cadmium induced testicular damage has been investigated in α-tocopherol (Vitamin E) pretreated and non-pretreated male rats exposed to a single sub-lethal dose of cadmium in form of $CdCl_2$. Graded doses of vitamin E (75, 150, and 750 mg kg^{-1} body wt.) were administered daily to rats in separate groups by gavage for 4 weeks while 3 mg Cd kg^{-1} body wt was administered subcutaneously, 24 hr to the termination of the study. Relative to the Cd - free control rats, cadmium significantly ($P < 0.05$) increased total cholesterol (CHL) levels in the testes and prostate but did not change its level in plasma. It also decreased TPL/CHL and phosphatidylcholine (PC) / phosphatidylethanolamine (PE) ratios in testes and increased sphingomyeline (SPM) / phosphatidylethanolamine (PE) ratios in the testes. However, cadmium administration increased the PC/PE and SPM/PE ratio but reduced the TPL/CHL ratio in the prostate. It appears that increased cholesterol levels within the testes and prostate and attendant membrane rigidity may be one mechanism by which cadmium causes damage to the testes and prostate. It also appears that low - medium doses of α-tocopherol can effectively protect the testes and prostate against Cd - induced damage.

Key words: Cadmium, α-tocopherol, cholesterol, phospholipids, rat.

INTRODUCTION

Most animals with scrotal testes are susceptible to cadmium – induced testicular toxicity (King et al., 1999). Although only about 1 - 2% of an acute cadmium dose is usually taken up by the testes, testicular toxicity is almost invariably evident. It has been reported that as low as 1 - 2 mg Cd kg^{-1} body wt. can cause testicular damage without pathological changes to other organs (Prozialeck et al., 2006). Exposure to cadmium has been reported to reduce male fertility in both humans and rodents (Benoff et al., 2000).

Several reports refer to the role of cholesterol in sperm capacitation and the acrosome reaction process (Khorasani et al., 2000). Hypercholesterolemia results in quantitative and qualitative alterations in sperm membrane lipids leading to an impaired sperm capacity for capacitation and acrosome reactions (Shimamoto and Sofikitis, 1998). In addition, these reports further revealed secretory dysfunction of stimulated leydig cells in a hypercholesterolemic environment.

Although cadmium is a well known testicular toxicant, its mechanism of toxicity on this organ has not been completely elucidated. Among the proposed mechanisms for its toxicity on the testes are; circulatory failure due to vascular damage and decreased utilization of Zn by spermatogenic cells due to competitive action of cadmium (Amara et al., 2008; Lee, Dixon, 1973). There is paucity of information on the effect of cadmium on the lipids of the testes. Consequently, there is a lack of information also on the role of cadmium - induced lipid changes in testicular function. The aim of the present study was therefore, to extend our investigation on the mechanism of cadmium-induced testicular toxicity by studying the lipid pattern in cadmium exposed and cadmium free rats and the effect of vitamin E, if any, on the lipid pattern.

MATERIALS AND METHODS

Thirty adult male albino rats (Wistar strain; 150 ± 20 g) purchased from the Laboratory Animal Unit of Lagos University Teaching Hospital (LUTH) were used in this study. The rats were allowed a 2 week acclimatization period. Thereafter, they were randomly divided into six groups of 5 rats each. They received water and chow (BFFM Ltd, Ewu, Nigeria) *ad libitum* throughout the period of the ex-

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periment. They were housed in cages with wire mesh floor (33 x 20 x 13) to prevent coprophagia. Rats in group 1 (control) were cadmium and vitamin E free (- Cd -Vit). Rats in group 2 received only cadmium (+Cd -Vit). Those in group 3 received only 750 mg vitamin E kg^{-1} body wt (-Cd+V750) while those in groups 4, 5 and 6 received 75, 150 and 750 mg vitamin E kg^{-1} body wt respectively before cadmium. Vitamin E was administered daily for 4 weeks by gavage but the cadmium in form of $CdCl_2$ dissolved in normal saline was administered to the rats at a dose of 3 mg kg^{-1} body wt (sc), 24 h to the termination of the experiment. These treatments were carried out in accordance with the principles of laboratory animal care (NIH publication no. 85 - 93, revised 1985). At the end of the 4 weeks experimental study period, animals were necropsized under chloroform (BDH, Poole, England) anesthesia. The thoracic and abdominal regions were opened and blood was obtained by cardiac puncture and put into heparinized tubes, for plasma preparation. The testes and prostate were excised, freed of connective tissue and weighed.

Extraction of lipids from testes

Total lipid was extracted from known weight of testes by the method of Bligh and Dyer (1959), as modified by Jensen (1976). After the removal of the tunica albuginea, the testes were homogenized in a mechanical homogenizer using 20 volumes of chloroform methanol mixture (2:1, v/v). The extract was filtered on a Buchner funnel through solvent-washed filter paper. Filter paper and residue were re-homogenized and extracted with the same chloroform methanol mixture, using half of the volume applied in the initial extraction. The filtration and re-extraction procedures were repeated once more, this time using chloroform methanol (1:2 v/v). The solvent composition of the combined extracts was adjusted by addition of chloroform and distilled water in order to attain a chloroform-methanol-water ratio of 16:8:1. The extract was then washed with 0.9% aqueous sodium chloride. The organic and aqueous layers were finally separated after centrifuging at low temperature (2000 rpm, 5°C). The washed extracts were concentrated in a rotary evaporator and the residue was dissolved in 2.0 ml chloroform: methanol mixture (2:1). At all stages during the extractions, the vessels containing lipid material were flushed with nitrogen.

Biochemical analysis

The amount of total lipid was determined by the method of Zollner and Kirsh (1962). In this method, lipids were allowed to react with sulphuric acid and phosphovanilin reagent to form a pink coloured complex which is measured spectophotometrically at 530 nm.

Total cholesterol was estimated in both the lipid extract and plasma by the enzymatic end-point method using commercially available kits (Randox , UK) while total phospholipids was determined by the method of Fiske and Subarrow (1925).

Individual phospholipid types were separated by thin layer chromatography (Curzner and Davidson, 1967). Spots corresponding to specific phospholipids were recovered by scraping and quantified by the method of Fiske and Subarrow (1925). Recovered phosphorlipid fractions and reference standards (1 - 5 µg P/ tube) were digested with perchloric acid. Digests were then incubated with molybdate and ascorbic acid solutions and the absorbance of the coloured complex formed was read at 800 nm.

Statistical analysis

Results were expressed as means ± (STD). Analysis of variance (ANOVA) was used to test for differences between treatment effects while Turkey multiple comparison tests was used to test for significant differences between the treatment means. Values were considered significant at $P < 0.05$.

RESULTS

Twenty four hours after dosing with Cd, testicular total cholesterol levels, were significantly ($p < 0.05$) increased in the groups that were treated with only Cd and both Cd and 750 mg vitamin E Kg^{-1} body wt (Table 1) when compared with the control group (-Cd –Vit). Also When testicular total cholesterol of the rats that were treated with only Cd were compared with those that were treated with both Cd and vitamin E, a significant ($p < 0.05$) reduction was observed only in the group that was treated with both Cd and 150 mg vitamin E Kg^{-1} body wt. Treatment with only 750 mg vitamin E kg^{-1} body wt (-Cd +V750) had a similar effect as cadmium on total cholesterol. In the prostate, total cholesterol of the rats that were treated with only Cd (+Cd-Vit), was also significantly ($p < 0.05$) higher than the control. However, pre-treatment of rats with vitamin E before Cd treatment reduced prostate total cholesterol to levels that were not significantly different from control. Treatment with the highest dose of vitamin E without cadmium (-Cd +V750) significantly increased total cholesterol levels in all the tissues investigated in this study when compared with the control. Table 1 also shows that cadmium did not have any significant ($p > 0.05$) effect on plasma cholesterol levels and total phospholipid levels of both testes and prostate, when compared with the control.

The phospholipid profile of the testes and prostate are shown in Tables 2 and 3. Cadmium significantly ($p < 0.05$) reduced phosphatidylcholine, phosphatidylethanolamine and sphingomyeline levels in testes when compared with the control. However, when compared with the group that was treated with only cadmium, rats that were treated with Cd and 75 mg vitamin E showed a significant ($p < 0.05$) increase in phosphatidylcholine level. A significant increase in sphingomyeline was observed in all the Cd and vitamin E treated rats when compared with the group that was treated with only cadmium. In the prostate, phosphatidylcholine and sphingomyeline were significantly reduced in rats that were treated with 75 and 150 mg vitamin E kg^{-1} body wt. when compared with the group that was treated with only cadmium. However, pre-treatment with 750 mg α-tocopherol before cadmium treatment, caused a significant ($p < 0.05$) reduction in the phosphatidylethanolamine levels in the prostate when compared with the group that was treated with only cadmium. When compared with the control, the group that was treated with only 750 mg vitamin E kg^{-1} body wt. showed a significant ($p < 0.05$) reduction in phosphatidylethanolamine level in the testes.

In this study, PC/PE, SPM/PE and TPL/CHL ratios were used as indexes of membrane fluidity so as to establish whether or not cadmium alters this important membrane phenomenon. The results presented in Table 4, show that cadmium decreased the TPL/CHL and PC/PE

Table 1. Effects of cadmium, α-tocopherol and α-tocopherol plus cadmium treatments on total cholesterol, total phospholipid and total phospholipid / cholesterol ratio in testes and prostate of rat.

Parameters	Groups					
	1 (-Cd -Vit)	2 (+Cd -Vit)	3 -Cd+V750	4 (+Cd+V75)	5 (+Cd+V150)	6 (+Cd+V750)
Testicular Total Cholesterol (CHL) (mg/ g tissue)	13.87 ± 1.34	32.36 ± 6.06 *	47.77 ± 5.73 *	21.46 ± 2.68	9.83 ± 0.22 a	37.57 ± 5.28 *
Prostate Total Cholesterol (CHL) (mg/ g tissue	42.50 ± 5.30	93.03 ± 9.45*	81.66 ± 3.60*	30.83 ± 4.43a	22.19 ± 1.60a	45.82 ± 4.84 a
Testicular Total Phospholipid (TPL) (mg /g tissue)	1.64 ± 0.22	1.41 ± 0.14	1.65 ± 0.22	1.26 ± 0.04	1.24 ± 0.12	1.45 ± 0.06
Prostate Total Phospholipid (TPL) (mg/ g tissue)	1.66 ± 0.19	1.46 ± 0.07	1.63 ± 0.11	1.25 ± 0.06	1.42 ± 0.08	1.45 ± 0.11
Plasma Total Cholesterol (PCHL) (mg / dl)	36.01± 3.37	37.31± 4.66	45.91 ± 2.63*	36.94 ± 3.60	37.70 ± 3.38	40. 23 ± 1.08

Values are ± SD (n = 5).
*Values on the same row followed by asterisks differ significantly (p< 0.05) from control.
a Values on the same row followed by an alphabet differ significantly (p<0.05) from the group that was treated with only cadmium.
-Vit - Cd = group was neither given vitamin E nor cadmium and served as the control.
- Vit +Cd = group was not given vitamin E but was treated with cadmium and served as the test control.
+ V750 - Cd = group received only750mg vitamin E and served as the vitamin control.
+ V75 + Cd = group was given 75mg vitamin E and administered cadmium.
+V150 + Cd = group was given 150mg vitamin E and administered cadmium.
+ V750 + Cd = group was given 750mg vitamin E and administered cadmium

Table 2. Effects of cadmium, α-tocopherol and α-tocopherol plus cadmium treatments on testes phospholipid profile.

Parameters	Groups					
	1(-Cd-Vit)	2(+Cd-Vit)	3(-Cd+V750)	4(+Cd+V75)	5(+Cd+V150)	6(+Cd+V750)
Phosphatidylcholine (PC) (mg/ g tissue)	6.44 ± 0.01	2.93 ± 0.01*	5.08 ± 0.02	7.50 ± 1.50 a	4.23 ± 0.12 *	4.06 ± 0.02 *
Phosphatidylethanolamine (PE) (mg/ g tissue)	5.30 ± 0.11	3.98 ± 0.04 *	4.30 ± 0.51 *	3.32 ± 1.00 *	2.32 ± 0.17*a	4.19 ± 0.31 *
Sphingomyeline (SPM) (mg/g tissue)	3.31± 0.50	1.47 ± 0.42 *	2.73 ± 0.27	3.98 ± 0.05 a	4.15 ± 0.16*a	3.02 ± 0.06a

Values are mean ± SD (n = 5).
*Values on the same row followed by asterisks differ significantly (p < 0.05) from control.
aValues on the same row followed by an alphabet differ significantly (p < 0.05) from the group that was treated with only cadmium.

Table 3. The effect of 4-weeks α-tocopherol pretreatment on cadmium induced changes in prostate phospholipid profile.

Parameters	Groups					
	1(-Cd-Vit)	2(+Cd-Vit)	3(-Cd+V750)	4(+Cd+V75)	5(+Cd+V150)	6(+Cd+V750)
Phosphatidylcholine (PC) (mg/ g tissue)	0.003 ± 0.000	0.005 ± 0.001	0.006 ± 0.001 *	0.003 ± 0.000 a	0.002 ± 0.000 a	0.009 ± 0.000*a
Phosphatidylethanol-amine (PE) (mg/ g tissue)	0.005 ± 0.000	0.004 ± 0.001	0.010 ± 0.001 *	0.005 ± 0.001	0.003 ± 0.000	0.002 ± 0.000 a
Sphingomyeline (SPM) (mg/g tissue)	0.005 ± 0.000	0.008 ± 0.001	0.005 ± 0.001	0.004 ± 0.001 a	0.003 ± 0.000 a	0.009 ± 0.002

Values are mean ± SD (n = 5).
*Values on the same row followed by asterisks differ significantly (p< 0.05) from control.
a Values on the same row followed by an alphabet differ significantly (p<0.05) from the group that was treated with only cadmium.
See table 1 footnote for other interpretations.

Table 4. The Effect of cadmium, α-tocopherol and α-tocopherol plus cadmium treatments on phosphatidylcholine (PC)/Phosphatidylethanolamine (PE), Sphingomyeline(SPM)/Phosphatidylethanolamine (PE) and Total phospholipid (TPL)/ Cholesterol(CHL) molar ratios in the testes and prostate of rat.

Organs	Parameters	Groups					
		1(-Cd-Vit)	2(+Cd-Vit)	3(-Cd+V750)	4(+Cd+V75)	5(+Cd+V150)	6(+Cd+V750)
Testes	PC/PE molar ratio	1.15	0.70	1.11	2.12	1.73	0.92
	SPM/ PE molar ratio	2.04	3.66	1.02	2.51	1.14	2.19
	TPL/CHL molar ratio	0.030	0.011	0.009	0.015	0.033	0.010
Prostate	PC/PE molar ratio	0.567	1.181	0.569	0.567	0.631	4.25
	SPM/ PE molar ratio	0.55	1.01	0.20	0.68	0.44	0.92
	TPL/CHL molar ratio	0.010	0.004	0.005	0.010	0.016	0.008

molar ratios but increased SPM/PE molar ratio in the testes when compared with the control. However, treatment with graded doses of α-tocopherol ameliorated these effects in the testes, with the 150 mg/kg body wt. dose being more effective. In the prostate, cadmium increased the PC/PE and SPM/PE ratios while the TPL/CHL ratio was reduced, when compared with the control. Treatment with 150 mg α-tocopherol Kg^{-1} body wt. increased TPL/CHL and PC/PE ratio but reduced SPM/PE ratio in the prostate.

DISCUSSION

The present study examined the effects of cadmium, and pre-treatment of rats with α-tocopherol before cadmium, on the ability of the toxicant to cause changes in the pattern of lipids in the testes and prostate.

Cadmium increased total cholesterol levels in the testes and prostate of rats. If this increase was due to dietary cholesterol, it would have reached the testes and prostate via the blood, thereby leading to a significant elevation in plasma cholesterol level. However, in this study, plasma cholesterol level was not significantly altered by cadmium in any of the rat treatment groups. Again it has been established that there is a blood – testes or blood – male reproductive tract barrier for cholesterol (Shimamato and Sifikitis, 1998; Wong et al., 2004). Evidently, the high testicular cholesterol level observed in this study cannot be attributed to diet. Rather, it is attributable to the intra - gonad alteration in lipid distribution; namely increased mobilization from the membrane of the cells within the testes and / or increased prostatic secretion of cholesterol into the seminal plasma in response to the presence of Cd within the gonad and accessory glands. The later view is more probable since cholesterol is normally secreted into the seminal plasma by the prostate (Sofikitis and Miyagawa, 1991) to protect the spermatozoa against environmental shock. The mechanism by which Cd stimulates increased cholesterol level in the prostate is not known. However, this metal causes oxidative stress in a number of tissues including the testes (Kara et al., 2007). It is therefore likely that secretion and build-up of cholesterol in the testes and prostate is a biological event that is meant to protect spermatozoa from oxidative stress and damage. The increase in cholesterol level in the testes of rats exposed to high doses of vitamin E only (Table 1, group 3) compared with the control rats is in agreement with earlier reports on increased liver (Alfin- Slater et al., 1972), brain (Dahl et al., 1974) and plasma (Mansour et al., 1992) cholesterol levels in rats and elderly men given high daily doses of α-tocopherol (Vitamin E). Pre-treatment with moderate dose of, 150 mg α-tocopherol kg^{-1} body wt was effective in reducing the high cholesterol levels in the testes and prostate. The presence of high level of cholesterol in the testes and prostate may be an indication of decreased androgen production by the testes. This is conceivably so because this hormone is produced by leydig cells (a group of cells that make up the testes) and the function of stimulated leydig cells are impaired by high cholesterol levels (Shimamoto and Sofikitis, 1998). The Cd-induced increase in cholesterol production as observed in this study will therefore affect negatively the leydig cell function. Optimal leydig cell function and testosterone secretion are known to be prerequisites for the normal activation of spermatogenesis (Hikim et al., 2005). Pre-treatment with low levels of vitamin E (75 and 150 mg/kg body wt) was effective in reducing cholesterol to levels close to that of the control while pre-treatment with high dose 750 mg kg^{-1} body wt. alone, or in combination with Cd enhanced cholesterol concentrations to levels higher than the control. This study reveals that the toxic effect of Cd in the testes may involve leydig cell dysfunction. Mechanistically, it could be caused indirectly by Cd when it elevates testicular and prostate cholesterol levels. However, moderate doses of vitamin E appear to be capable of obviating this effect of Cd on testicular cholesterol level.

Total phospholipids / cholesterol (TPL/CHL), phosphatidylcholine/ phosphatidyl ethanolamine (PC/PE) and sphingomyeline / phosphatidyl ethanolamine (SPM / PE) molar ratios are known indexes of membrane fluidity (Bangur et al., 1995). Increase in the PC/PE and SPM/PE

molar ratios and a decrease in the TPL/CHL molar ratio indicate decreased membrane fluidity (Bangur et al., 1995).

In this study, PC/PE, SPM/PE and TPL/CHL ratios were used as indexes of membrane fluidity so as to establish whether or not cadmium alters this important membrane phenomenon. The study shows that cadmium decreased the TPL/CHL and PC/PE molar ratios but increased SPM/PE molar ratio in the testes. Increase in both PC/PE and SPM/PE molar ratios should indicate decreased membrane fluidity but this study shows a decrease in PC/PE and an increase in SPM/PE ratios. Since the two ratios counterbalance each other, the net effect is that cell membranes in the testes will be neither completely fluid nor completely rigid in the presence of cadmium. In the prostate, the PC/PE and SPM/PE ratios were increased while the TPL/CHL ratio was reduced. These effects on prostate phospholipid ratios will bring about complete decrease in its membrane fluidity. The contrasting effects of this toxicant on the testes and prostate lend credence to the suggestion that the effect of Cd on tissue phospholipid may be organ dependent. This effect on the testes and prostate phospholipid ratios was not generally reversed in rats exposed to 75 and 750 mg vitamin E kg^{-1} bd wt., respectively, before cadmium intoxication. In terms of PC/PE ratio, pretreatment of rats with lower doses of vitamin E (75 and150 mg vit. E kg^{-1} bd wt. respectively) before Cd exposure improved membrane fluidity in the testes and prostate. However, if only SPM/PE and TPL/CHL were used to determine membrane fluidity, this membrane phenomenon will be increased in the testes and prostate when Cd – intoxication is preceded by vitamin E treatment at a dose of 150 mg kg^{-1} bd. wt. Therefore, an increase in TPL/CHL ratio and a corresponding decrease in SPM/PE ratio increase membrane fluidity in the presence of Cd rather than the decrease observed in rats exposed to Cd alone. This effect of Cd on the testes is similar to that reported by Pandya et al. (2004) that long term exposure to aluminium causes an increase in cholesterol level and a decrease in TPL/CHL ratio in the brain of rats.

In conclusion, the mechanism of cadmium toxicity of the testes and prostate may involve elevation of cholesterol levels in these organs. Evidently, moderate doses of vitamin E protect the testes and prostate from Cd – induced alteration in membrane fluidity while high doses of the vitamin, when administered alone has effect similar to Cd on the testes.

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