The Protective Role Of Ascorbic Acid Administration On The Bicalutamide Induced Reduction In Semen Quality In The Sprague - Dawley Rat.

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Citation

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Abstract

Antineoplastic drugs used in the treatment of testicular carcinoma have been reported to have deleterious effects on the fertility potentials in affected males. The mechanism of damage involves the generation of free radicals.We evaluated the effect of using an antioxidant ascorbic acid as an adjuvant treatment with Bicalutamide on the androgen sensitive organs of the male Sprague-Dawley rats.Thirty-five adult male Sprague-Dawley rats weighing between 160-180 g were used for the experiment. The animals were randomly selected and divided into seven groups of five rats each. They were given 10 mg/kg or 20 mg/kg body weight of Bicalutamide and 200 mg/kg of Ascorbic acid for three and eight weeks. The animals were sacrificed and the cauda epididymides excised for sperm count and sperm motility analysis.There was observed decrease in semen quality with Bicalutamide that was attenuated by ascorbic acid co-administration. The concomitant administration of ascorbic acid has a protective role on reproductive function in males on Bicalutamide therapy.

INTRODUCTION

Bicalutamide is used for the treatment of prostatic and testicular cancers. These two forms of androgen specific cancers have an increasing incidence among the adult male populace (Potosky et al., 1995; Hans et al., 1996; Huvghe et al., 2003). Drugs used in chemotherapy of these malignancies adversely affects the reproductive function and may lead to infertility where there is still the desire to procreate (Robbins et al., 1998). Testicular cancer affects the reproductive age group with a peak age of incidence between 20 – 34 years (Huvghe et al., 2003). Though the incidence of prostatic carcinoma varies significantly across ethnic groups, peoples of African descent are at the highest risk (Alice et al., 1995; Parkin et al., 1999). The male reproductive organ malignancies are androgen sensitive having androgen receptors on their cellular membrane surfaces and therefore can be controlled or treated by the administration of anti androgens (Olea et al., 1990; Naohide et al., 1999).

In Nigeria there is a burden of androgen sensitive tumours particularly that of the prostate. The incidence of prostatic carcinoma has been reported in literature to be on the increase (Ogunbiyi and Shittu, 1999; Ekwere and Egbe 2002). Some authors have reported that prostate cancer is the most commonly diagnosed cancer among male Nigerians (Ann and Susan, 2001). The incidence of testicular cancer on the other hand remains low in Nigeria (Magoha, 1995; Izegbu et al., 2005); the early age of onset however raises concern about future reproductive potentials of those affected (Stone et al., 2005).

Infertility is an important medical problem of great social and cultural dimensions in Sub Saharan Africa, with several figures reported in literature for the Nigerian population (John and Pat, 1987; Robert et al., 1993).

Anti-androgens either from the environment or iatrogenically administered are endocrine disruptors (Kelce and Wilson 1997; Jane, 2004) have deleterious effect on male reproductive function (Rex, 1998; Gray et al., 2001). Bicalutamide is an anti-androgen used in the chemotherapy of androgen sensitive tumours (Michael, 2002). The mode of action is by preventing testosterone from binding to the androgen receptors on the prostate and testicular cancer cells. It therefore starves the cancer cell of testosterone, which prevents their growth. Eventually the prostate and the testicular tumor will shrink (Amory, 2007).

With the benefit of slowing down the tumor growth, it has also been implicated in the development of male infertility among those on treatment with the drug (Amory, 2007).

Use of Antioxidants as agents to scavenge free radicals and reducing oxidative stress in reproductive organs is justifiable as increased oxidative stress has been shown to impair male reproductive function (Sikka et al., 1995; Sharma et al., 2001). The potential role of antioxidants, such as ascorbic acid, tocopherol, B-carotene, etc., to reduce the activity of free radical-induced reactions has drawn increasing attention (Chakraborty et al., 1994; McCall, 1999).

In this study we investigated the effect of antioxidant ascorbic acid on semen parameters of male Sprague-Dawley rats treated with anti androgen Bicalutamide.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Thirty-five adult (8-10 weeks old) male Sprague-Dawley rats weighing between 160-180g were used for the experiment. They were procured from the animal house center of the College of Medicine University of Lagos. The animals were randomly divided into seven groups of five rats each. They were housed in well ventilated metal cages in the rat room of the department of Anatomy, College of Medicine, University of Lagos under standard conditions and 12: 12 light: dark photoperiodicity. Ambient temperature was between 28°C – 33°C. The animals had access to rat chow and water ad libitum. The care of the animals was in accordance with the national law on animal care and use (Zimmerman, 1983).

BICALUTAMIDE AND ASCORBIC ACID

The drugs were purchased from the pharmacy department of the Lagos University teaching hospital. Bicalutamide (Casodex®) was in tablet form; the tablets were crushed and completely dissolved in water.

THE EXPERIMENTAL PROTOCOL IS AS FOLLOWS:

Group A: received 10 mg/kg body weight of Bicalutamide daily for 3 weeks

Group B: was administered 10 mg/kg body weight of Bicalutamide daily for 8 weeks

Group C: control, was given equal volumes of distilled water per kg body weight for 3 and 8 weeks.

Group D: Had 20 mg/kg body weight of Bicalutamide for 3 weeks

Group E: Had 20 mg/kg body weight of Bicalutamide for 8 weeks

Group F: Had 10 mg/kg body weight of Bicalutamide and 200 mg/kg body weight of ascorbic acid for 3 weeks

Group G: Had 20 mg/kg body weight of Bicalutamide and 200 mg/kg body weight ascorbic acid for 8 weeks

The route of administration of test substances in all the rats was by gavage.

ANIMAL SACRIFICE AND SEMINAL FLUID ANALYSIS

At the end of the experimental period, the rats were sacrificed by cervical dislocation and at necropsy the cauda epididymides of the rats were removed. Several small cuts were made and then placed in 1 ml of normal saline and spermatozoa allowed swim up and the supernatant analyzed for count and motility studies (Yan et al., 2007; Akang et al., 2010).

STATISTICAL ANALYSIS

The statistical analysis was carried out using student's t- test at $p < 0.05$.

RESULTS

The results showed significant decrease in mean sperm count from control values of 81.8 ± 1.06 in all the treatment groups; there were similar observed decreases in mean sperm motility.

Figure 1

TABLE 1: Mean sperm count (Millions/ml)

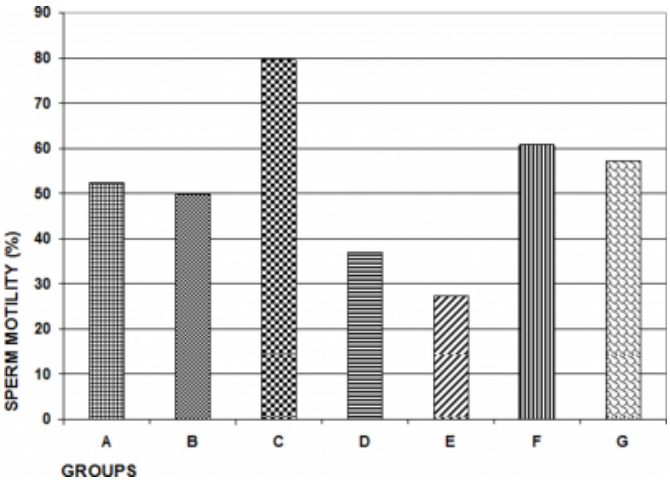
Groups	Drug Administration	Mean sperm count (x 10^6/ml) ± SEM
A	10 mg/kg of Bicalutamide (3 wks)	67.8 ±2.13*
B	10 mg/kg of Bicalutamide (8 wks)	65.4 ±2.20*
C (Control)	Distilled water (3 and 8 wks)	81.8 ± 1.06
D	20 mg/kg of bicalutamide (3 wks)	60.8 ±2.15*
E	20 mg/kg of Bicalutamide (8 wks)	50.4 ±3.14*
F	10 mg/kg of Bicalutamide and 200 mg/kg of Ascorbic acid (3 wks)	77.8 ±1.65**
G	20 mg/kg of Bicalutamide and 200 mg/kg of Ascorbic acid (8 wks)	75.2 ±1.62**

*Significant at p<0.05

S.E.M: Standard Error of Mean

Figure 2

FIGURE 1: Showing percentage Sperm motility of the different groups



There were statistically significant decrease groups in groups B and D. p< 0.05.

DISCUSSION

The result of the sperm counts in millions per milliliters at p<0.05 shows a statistically significant reduction in the count and a concomitant reduction in percentage sperm motility in groups B and D. This result is in consonance with previously reported deleterious effects observed in semen parameters in rats treated with Bicalutamide. Bicalutamide is an endocrine disruptor (Kelce and Wilson, 1997; Jane, 2004) and as has been shown with other endocrine disruptors, it has deleterious effect on reproductive function of male Sprague-Dawley rats. The groups treated with concomitant ascorbic acid administration did not show any statistically significant difference when compared with the control (Table 1 and Figure 1). Ascorbic acid administered as an adjunct modulates the deleterious effects of Bicalutamide. Ascorbic acid, a water soluble vitamin is well known for its anti-oxidant properties (Chakraborty et al., 1994; Glenn et al., 1996; McCall and Balz, 1999;). As an anti-androgen Bicalutamide therapy causes cellular androgen deprivation. It removes the testosterone induced augmentation of basal reactive oxygen species production with resultant diminution of the cellular response against oxidative stress. The diminished production of reactive oxygen species (ROS) ablates or at least diminishes cellular response to oxidative stress which ascorbic acid restores (Winkler 1999; Jehonathan et al., 2007). This has proven beneficial in this experiment. High concentration levels of free radicals are known to be hazardous to living organisms and lead to

damage of all major cellular constituents. At moderate concentrations however, ROS play an important role as regulatory mediators in the signaling processes. Many of the ROS-mediated responses actually protect the cells against oxidative stress (Oschsendorf, 1999; Droge, 2002).

Ascorbic acid is also reported to have empirical effects on the reproductive system; it has been shown that ascorbic acid supplement maintains male fertility even when administered empirically (Sapra et al., 1987).

CONCLUSION

Administration of Ascorbic acid as an adjuvant in anti-androgen therapy would prove beneficial, particularly in the maintenance of semen quality parameters of sperm count and motility. The protective effect of ascorbic acid may be of clinical benefit as an adjunct treatment of males with androgen sensitive tumors on Bicalutamide therapy who may wish to preserve their reproductive function.

AKNOWLEDGEMENT

The authors are grateful to the family of Late Samuel Adewale Solarin for funding the procurement of the Bicalutamide (Casodex®) used in the experiments. Furthermore, the use of the animals was approved by the Experimental Ethics Committee on Animals Use of College of Medicine, University of Lagos, Nigeria.

References

r-0. Akang, E. N., Oremosu, A. A., Dosumu, O. O., Noronha, C. C. and Okanlawon, A. O. The effect of fluted pumpkin (Telfairia occidentalis) seed oil (FPSO) on testis and semen parameters. ABJNA. 1 (2010), pp. 697-703
r-1. Alice SW, Laurence NK, Anna HW, Esther MJ, Richard PG, Geoffrey RH, David BJ, Jean H, Darlene MD, Dee WW, Chong-Ze T, Ralph SP. Prostate Cancer in Relation to Diet, Physical Activity, and Body Size in Blacks, Whites, and Asians in the United States and Canada. J. Natl Cancer Inst; 1995; 87:652-661.
r-2. Amory JK. Drug effects on spermatogenesis. Drugs Today 2007; 43: 717.
r-3. Ann WH , Susan SD . Trends and Patterns of Prostate Cancer: What Do They Suggest? Epidemiol Rev; 2001; 23: 3-13
r-4. Chakraborty S, Nandi A, Mukhopadhyay CK, Chatterjee IB. Ascorbate protects guinea pig tissues against lipid peroxidation. Free Rad BiolMed 1994; 16:417-426.
r-5. Dröge W. Free radicals in the physiological control of cell function. Physiol Rev. 2002; 82:47-95.
r-6. Ekwere PD, Egbe SN. The changing pattern of prostate cancer in Nigerians: current status in the southeastern states. J.Natl Med Assoc. 2002; 94:619-627.
r-7. Glenn NL, Balz F, Spyridon NK, Marie DG, John FK, Joseph AV, Ascorbic Acid Reverses Endothelial Vasomotor Dysfunction in Patients With Coronary Artery Disease Circulation. 1996; 93: 1107-1113.
r-8. Gray LE, Ostby J, Furr J, Wolf CJ, Lambright C,. Parks L, Veeramachanen DN Wilson V, Price M, Hotchkiss A, Orlando E, Guillette L.> Effects of environmental antiandrogens on reproductive development in experimental animals. Hum Reprod Update 2001; 7: 248-264.
r-9. Hans-Olov A, Matthias M, Witold Z, Hans, Anders, Steinar, Lyly, Olof, Timo H. Increase in Testicular Cancer Incidence in Six European Countries: a Birth Cohort Phenomenon Reinhold Bergström. J. Natl Cancer Inst; 1996; 88:727-733.
r-10. Huyghe E, Matsuda T, Thonneau P. Increasing incidence of testicular cancer worldwide: a review. J Urol. 2003; 170:5-11
r-11. Izegbu MC, Ojo MO, Shittu Clinico-pathological patterns of testicular malignancies in Ilorin, Nigeria-a report of 8 cases. JCRT; 2005; 1: 229-231.
r-12. Jane SF. Environmental anti-androgens and male reproductive health: focus on phthalates and testicular dysgenesis syndrome . Reproduction; 2004; 127: 305-315
r-13. Jehonathan HP, Inna Bryskinyv YZ, John T, Jiang-Ping L, Gurmit S, Eduard F, Brian CW. Neoplasia Androgen Induces Adaptation to Oxidative Stress in Prostate Cancer: Implications for Treatment with Radiation Therapy1 2007; 9: 68 – 80.
r-14. John CC, Pat C. The Cultural Context of High Fertility in sub-Saharan Africa. Population Devel Rev; 1987; 13: 409-437
r-15. Kelce R, Wilson EM. . Environmental antiandrogens: developmental effects, molecular mechanisms, and clinical implications . J Mol Med 1997; 75: 198-207.
r-16. Magoha GA. Testicular cancer in Nigerians. East Afr Med J; 1995; 72:554-556.
r-17. McCall MR, Balz F. Can antioxidant vitamins materially reduce oxidative damage in humans'7 Free Rad Biol Med 1999; 26: 1034 - 1053.
r-18. Michael KB, Urology Update from Jackson Hole Highlights from the 3rd Annual Jackson Hole Summer Urologic Conference July 28– Rev Urol. 2002; 4: 78–86.
r-19. Naohide S, Martin EG,, Nicholas B, Paul SR, Larry SG, Paul HL, Lorne DS. Intermittent androgen suppression delays progression to androgen-independent regulation of prostate-specific antigen gene in the LNCaP prostate tumour model. J Steroid Biochem Mol Biol; 1996; 58: 139-146
r-20. Ogunbiyi JO, Shittu OB. Increased incidence of prostate cancer in Nigerians. J Natl Med Assoc; 1999; 91:159-64.
r-21. Olea,N., Sakabe, K, Soto, AM and Sonnenschein, C. The proliferative effect of “anti-androgens” on the androgen-sensitive human prostate tumor cell line LNCaP. Endocrinology. 1990; 126: 1457-1463.
r-22. Oschsendorf FR. Infections in the male genital tract and reactive oxygen species. Human Reproduction Update 1999; 5: 399-420.
r-23. Parkin D, Pisani P, Ferlay J. Estimates of the worldwide incidence of 25 major cancers in 1990. Int. J. Cancer 1999; 80: 827-841.
r-24. Potosky, B.A., Miller,P.C., Albertsen A, Kramer, B.S. The role of increasing detection in the rising incidence of prostate cancer. J. Am. Med Ass. L. 1995; 273: 548–552
r-25. Rex AH. Effects of environmental toxicants on the efferent ducts, epididymis and fertility. J Reprod Fertil Suppl 1998; 53: 247-259.
r-26. Robbins AS, Whittemore AS, Van Den Eeden SK. Race, prostate cancer survival, and membership in a large health organization. J. Natl. Cancer Inst; 1998; 90: 986-990.
r-27. Robert JI, Leke, Jemimah AO, Bassol-Mayagoitia S, Bacha AM, Kenneth MG. Regional and Geographical Variations in Infertility: Effects of Environmental, Cultural,

and Socioeconomic Factors - Impact of the Environment on Reproductive Health. Environ Health Perspect; 1993; 101: 73-80
r-28. Sapra MM, Sharma PP, Kothari LK. The effect of Vitamin C deficiency on the testicular structure in the guinea pig. Postgrad Med.1987; 33:69-73.
r-29. Sharma RK, Pasqualotto FF, Nelson DR, Thomas AJ, Agarwal A. Relationship between seminal white blood cell counts and oxidative stress in men treated at an infertility clinic. J.Androl 2001; 22: 575 – 583.
r-30. Sikka SC, Rajasekaran M, Hellstrom WJ. Role of oxidative stress and antioxidants in male infertility. J Androl. 1995; 16: 464-468.
r-31. Stone JM, Cruickshank DG, Sandeman TF, Matthews JP. Trebling of the incidence of testicular cancer in Victoria, Australia (1950-1985). Cancer; 1991; 68:211-219.
r-32. Winkler BS. Unequivocal evidence in support of the non enzymatic redox coupling between glutathione/glutathione disulfide and ascorbic acid/dehydroascorbic acid. Biochim Biophys Acta; 1999; 1117: 287-90.
r-33. Yan J., Agresti M., Bruce T., Yan Y., Granlund A., Matloub H. Effects of cellular phone emissions on sperm motility in rats. Fertility and Sterility; 2007, 88: 957-964
r-34. Zimmerman M., Ethical guidelines for investigation of experimental pain in conscious animal. Pain; 1983; 16: 109-110

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