Academic Sciences Asian Journal of Pharmaceutical and Clinical Research
Vol 4, Suppl 2, 2011 ISSN - 0974-2441

Research Article

ALTERATIONS IN MORPHOMETRY AND MALONDIALDEHYDE LEVELS IN ADULT SPRAGUE-DAWLEY RAT TESTES IN THREE OBSTRUCTIVE VASECTOMY MODELS: EFFECT OF MELATONIN

F.I.O. DURU, O.E. YAMA*, C.C. NORONHA, A.O. OKANLAWON

Department of Anatomy College of Medicine, University of Lagos, Idi-Araba, Lagos, Nigeria, Email: dro_yama@yahoo.com

Received: 20 August 2011, Revised and Accepted: 27 September 2011

ABSTRACT

Vasectomy is usually offered as irreversible male contraception. The procedure which induces testicular oxidative stress leads to increased membrane peroxidation and alterations in testicular structure may impair reproduction even if vas deferens patency is re-established. The objective of the study is to investigate the alterations in the testicular morphometric and malondialdehyde (MDA) parameters following vasectomy at three different sites and the corresponding effect of melatonin. Eighty adult male Sprague-Dawley rats were randomized into four equal groups. The control group underwent right sided orchidectomy. The parameters evaluated include testicular histomorphometry (quantitative study of the microscopic organization and structure of a testicular tissue) in addition to estimation of baseline MDA levels. In the second group ligation was applied between the *caput epididymis* and upper pole of the testis. In the third group the right vas deferens was divided 2-3 cm from the *caudal epididymis* and both ends ligated, and in the fourth group, only the proximal end of the divided vas deferens was ligated. In addition, five animals in each group received 1mg/kg melatonin intra-peritoneally. After 12 weeks, both testes in each experimental animal were removed for MDA estimation and testicular histomorphometry. The closer the point of ligation is to the testis, the greater the testicular MDA level. The least MDA level was obtained when ligation was applied only to the proximal end of the sectioned vas deferens. Melatonin significantly reduced the MDA level in the testis for all the vasectomy sites. Ligation of the vas deferens did not affect the testicular diameter and mean seminiferous tubular diameter of the contralateral testis. In conclusion vasectomy induces oxidative stress in the ipsilateral testis which may be affected by the distance between the testis and the site of vasectomy. Melatonin reduces the testicular damage following vasectomy.

Keywords: Vasectomy, malondialdehyde, melatonin, oxidative stress

INTRODUCTION

Vasectomy is the surgical ligation and division of the vas deferens carried out as male contraception. It is a widely accepted form of contraception especially in India, China, United States and new-Zealand[1, 2]. It is usually regarded as a permanent surgical procedure with no assurance of successful reversal as far as reproductive function is concerned[3, 4]. There are some variations on the procedure such as no-scalpel (keyhole) vasectomies, in which a surgical hook, rather than a scalpel, is used to enter the scrotum[5, 6]. Another type of vasectomy which may reduce the risk of chronic pain could be the so-called "open ended" vasectomy which seals only the top end of the vas. With this method sperm are free to spill out from the lower severed end of the vas thus avoiding any build-up of pressure. The likelihood of long-term testicular pain from "backup pressure" seems to be eliminated using this method[5, 6]. Therefore factors considered in making the choice of the type of vasectomy include post-procedure morbidity, side effects and choice of the client[7, 8]. Irrespective of the procedure, the ultimate aim of vasectomy is to prevent the ejaculation of spermatozoa following coitus. However, it has been observed by several investigators that following vasectomy, the testes soon begin to undergo structural damage[9, 10, 11].

Evidence for post-vasectomy testicular pathology includes testicular atrophy, destruction of epithelium of the seminiferous tubules, absence of spermatozoa, thickening and folding of the tubular membranes and dilatation of the interstitial space[12]. Other abnormalities described include testicular interstitial fibrosis[13], enhanced apoptosis of spermatogenic cells[14] and morphological degeneration in the sperm tail and head[15], *epididymal* distension and sperm granuloma formation[16] with pathological changes in the vas deferens proximal and distal to the vasectomy site[17]. There are also data to show increased lymphocyte activity and increased anti-sperm antibody production[18], increased pressure of distal *epididymis*, increased size and number of lysosomes in *epididymis* and chronic inflammation of *epididymal* interstitium[19]. The incidences of testicular and prostate cancer have been reported to increase following vasectomy[20, 21] though other reports have opposed this view[22, 23]. The testicular damage following vasectomy is not uniform, but depends on the animal species, technique of vasectomy and the time interval after vasectomy[24]. Several mechanisms have been proposed as being causally related to the testicular damage following vasectomy. These include increased hydrostatic pressure in the testis, increased production of anti-sperm auto-antibodies, and oxidative stress resulting from increased production of reactive oxygen or nitrogen species[25,26]. Increased hydrostatic pressure in the testes is a more plausible explanation [10]. Conversely anti-sperm antibodies do not seem to play the same role on all species studied so far. In the rabbit it does not seem to be associated with testicular damage following vas deferens obstruction[27], whereas in the rat, its production precedes the testicular damage[18].

Most men who have chosen vasectomy as the choice of contraception value the freedom from artificial birth control and the fear of unplanned pregnancy. However, some people come to regret the operation and want it reversed, usually because they have a new partner or want more children[28, 29]. There is paucity of data on the precise cause of testicular damage following vasectomy and the possibility of reversal of the testicular damage. Preventing or reversing the testicular damage following vasectomy may imply a more favorable outcome in those patients asking for vasectomy reversal.

MATERIALS AND METHODS

Animals

The study comprised 80 adult male Sprague-Dawley rats (S-D) obtained from the Laboratory Animal Centre of the College of Medicine of the University of Lagos. They were authenticated by a taxonomist[30] in the Department of Zoology of the University of Lagos. Animals were kept in metal cages in the Animal Room of the Department of Anatomy and allowed to acclimatize for two weeks under standard laboratory conditions of temperature 27-30^{o}C, with a photoperiodicity of approximately twelve hours light alternating with twelve hours of darkness. They were fed with commercially available rat chow (Livestock feeds Plc., Ikeja, Lagos Nigeria) and had unrestricted access to water.

Experimental protocol

The experimental rats were divided randomized into four groups (I to IV) of 10 S-D rats per group. They were made up of a sham operated group and three different obstructive vasectomy models. Group I: Intact rats; Group II: Ligation applied between *caput epididymis* and upper pole of the testis; Group III: Ligation applied to both ends (proximal and distal) after division of vas deferens 2-3 cm from *caudal epididymis*; Group IV: Vas divided 2-3 cm from *caudal epididymis* and ligation applied only to the proximal end. In addition, five animals in each group received 1 mg/kg melatonin intra-peritoneally.

Each experimental group was controlled matched with 10 S-D rats that underwent right sided orchidectomy used for the estimation of baseline testicular malondialdehyde levels and cytometry (measurement of cells and cellular constituents). After 12 weeks, the right testes were removed for MDA estimation, histological processing for testicular morphometric measurements.

Anaesthesia and the surgical procedure

The anaesthesia was administered by an intra-peritoneal injection of 50 mg/kg ketamine hydrochloride. All operations were carried out on the right side through a scrotal incision. In the sham operated group, the testes and spermatic cord were exposed and closed. In the vasectomised groups, ligation was applied between the *caput epididymis* and upper pole of the testis with 2/5 silk sutures, or the right vas deferens was divided 2-3 cm from the *caudal epididymis* and both ends ligated, and in the last group, only the proximal end of the divided vas deferens was ligated.

Determination of malondialdehyde

Testicular malondialdehyde (MDA) levels were determined using the modified thiobarbituric acid (TBA) method of Buege and Aust[31], and the results expressed in µmol/mg protein. MDA reacts with thiobarbituric acid to give a red compound absorbing at 535nm. The stock reagent contains 2ml 15%w/v trichloroacetic acid, 0.375%w/v thiobarbituric acid and 0.25 mol/L hydrochloric acid, warmed if necessary to dissolve the thiobarbituric acid. 0.5 g testicular tissue sample was homogenized in 5 ml of 0.15 MKCl and the homogenate centrifuged at 1000 g for 10 min in a Uniscope laboratory centrifuge and the supernatant collected. An aliquot of 2 ml of the stock reagent was added to 1 ml of testicular homogenate supernatant and mixed thoroughly and placed in an Equitron water bath (80-90^{o}C) for 15 min. It was then cooled and the flocculent precipitate removed by centrifugation at 1000 g for 10 min and the absorbance of the supernatant determined with a Spectronic spectrophotometer at 535 nm against blank containing all the reagents. Concentration of malondialdehyde was calculated using the molar absorptivity coefficient of MDA which is $1.56 \times 10^5\ M^{-1}cm^{-1}$.

Testicular morphometry

The testes were carefully dissected out, trimmed of all fat and blotted dry to remove any blood. They were fixed in Bouin's fluid for 72 hours and then dehydrated by channeling through graded concentrations of alcohol. The tissues were cleared in chloroform and finally embedded in paraffin wax at 58^{0}C to 60^{0}C. Tissues were sectioned at 5 µ thickness. Cut sections were mounted on glass slides after treating tissues with egg-albumen and stained with Haematoxylin and Eosin.

Tissue specimens were examined for estimation of mean seminiferous tubular diameter (MSTD). The MSTD was calculated by measuring the smallest diameter of 10 randomly selected fields[32] using an ocular micrometer at x400. The results were then analyzed.

Statistical analysis

All data were expressed as mean ± standard deviation. Analysis was carried out using analysis of variance (ANOVA) with Scheffe's post hoc test. The level of significance was considered at $p < 0.05$.

RESULTS

Testicular malondialdehyde levels in Sprague-Dawley rats

In all the groups, ligation of the vas deferens caused a significant increase in MDA when melatonin was not given ($p < 0.05$). Testicular MDA increased as the point of ligation got closer to the testis. At each point of ligation, the testicular MDA was significantly reduced if melatonin was administered ($p < 0.05$). Testicular MDA was least when only the proximal end of the sectioned vas deferens was ligated (Table 1).

Table 1: Malondialdehyde levels in adult rat testes (µmol/mg protein) in three obstructive models of the vas deferens ± Melatonin (Mel)

Groups	(-Mel)	(+Mel)
I	0.38±0.24	0.38±0.07
II	0.92±0.16	0.47±0.23
III	0.69±0.02	0.42±0.14
IV	0.40±0.21	0.38±0.02

I - Intact rats
II- Ligation applied between *caput epididymis* and upper pole of the testis
III- Ligation applied to both ends after division of vas-deferens 2-3 cm from *caudal epididymis*
IV- Vas divided 2-3 cm from the *caudal epididymis* and ligation applied only to the proximal end

Testicular Morphometry in Sprague-Dawley rats

The diameter of the right testis was significantly reduced in all experimental groups compared to the controls ($p < 0.05$). Ligation of the vas deferens of the right testes did not significantly affect the diameter of the corresponding contralateral testis. Ligation of the vas deferens caused a significant decrease in mean seminiferous tubular diameter in all experimental groups ($p < 0.05$). The seminiferous tubular diameter was least affected when only the proximal end of the divided vas was ligated. Mean testicular tubular diameter was not affected in the intact left testis in all experimental groups (Table 2).

Table 2: Bilateral testicular diameters and mean seminiferous tubular diameter (MSTD) following three obstructive models of the vas deferens

	Test groups			
	I	II	III	IV
Number of rats (n)	5	12	12	12
Mean diameter (mm) Right testis	15.8	11.2	12.8	14.6
Mean diameter (mm) Left testis	15.2	14.8	15.0	15.4
MSTD (µm) Right testis	198.2	180.3	178.6	176.4
MSTD (µm) Left testis	196.2	194.7	198.2	198.5

I - Intact rats
II - Ligation applied between *caput epididymis* and upper pole of the testis
III - Ligation applied to both ends after division of vas-deferens 2-3 cm from *caudal epididymis*
IV- Vas divided 2-3 cm from the *caudal epididymis* and ligation applied only to the proximal end

DISCUSSION

Depending on the site of blockage, congenital and acquired obstruction of the male genital duct causes an increase in hydrostatic pressure in the seminiferous tubules, rete testis, efferent ductules, *epididymal* duct and the proximal part of the vas deferens[33]. The secretory epithelium of the male ductal system tends to degenerate in response to obstruction[34]. In this study, the diameters of the testes in the vasectomised rats were significantly decreased especially when the vasectomy site was closest to the testis; however there was no significant effect on the diameter of the contralateral testis in all the groups. This is in agreement with the observations of some authors[10], though other authors have reported more severe contra-lateral testicular injury in the mature rat[35]. The

testicular damage is believed to result from both increased intra-testicular pressure and the production of anti-sperm auto-antibodies as well as oxidative stress. This results from increased production of reactive oxygen or nitrogen species[25,26]. An amplified hydrostatic pressure is believed to be more important in the pre-pubertal than the adult rat, and is suspected to be due to the developed collateral circulation, and the rat testicular microcirculation with a rhythmic pattern of arteriolar dilatation and constriction[10] demonstrated in the mature rat. Anti-sperm antibodies though outside the scope of this study do not seem to have the same significance in all species studied so far. In the rabbit it does not appear to be associated with testicular damage following vasal obstruction[36], whereas in the rat, its production precedes the testicular damage[18]. That melatonin was able to reduce the MDA levels in all the groups studied suggest that free radicals play a role in testicular damage following vas deferens obstruction. Our data suggest that the more proximal the ductal obstruction, the more the testicular lipid peroxidation. A proximal obstruction of a secretory organ will more likely lead to an increase in intra-organ hydrostatic pressure than a distal obstruction. In the group with ligation of only the proximal end of the divided vas deferens, the MDA level was least, suggesting that lipid peroxidation is reduced if the drainage of the vas is maintained. In one study[37] it was reported that open-ended vasectomy did not affect testicular structure in a six month period. It is likely that increased intra-luminal pressure may be the primary cause of changes in the testis, because when the testicular end of the duct is left open, with drainage of the seminiferous tubules, abnormal changes were not observed in the testicular MDA level. This agrees with several investigators who used morphometric parameters to assess testicular damage following different vasectomy protocols[38,39]. We conclude that vasectomy alters ipsi-lateral testicular structure and increases lipid peroxidation and that melatonin may prevent this biochemical change. However further studies in the human are recommend as there are species differences in the response of testicular structure to vasectomy[40].

REFERENCES

1. Lohiya NK, Manivannan B, Mishra B, Pathak N. Vas deference, a site for male contraception: an overview. Asian J. Androl. 2001; 3 87-95.
2. Sneyd MJ, Cox B, Paul C, Skegg DC. H.igh prevalence of vasectomy in New-Zealand. J. Contracep. 2001; 64(3) 155-9.
3. Shapiro RH, Muller CH, Chen G, Berger RE. Vasectomy reversal associated with increased reactive oxygen species. J. Urol. 1998; 160(4):1341-6.
4. Fuchs EF, Burt RA. Vasectomy reversal performed 15 years or more after vasectomy; correlation of pregnancy results of in vitro fertilization with intracytoplasmic sperm injection. J. Fertil. Steril. 2002; 77(3): 516-9.
5. Huber HD, Krieger JN. No-scalpel vasectomy at the King's birthday vasectomy festival. A Nirapathpongporn. Lancet, 1990; 335: 894-895.
6. EurekaAert 2007. No-scalpel vasectomies by skilled surgeons may speed recovery. http://www.eurekalert.org/pub_releases/2007-04/cfta-nvb041707.php.
7. Holt BA, Higgins AF. Minimally invasive vasectomy. Brit. J. Urol. 1996; 77 (4): 585-6.
8. Skriver M, Skovsguard F, Miskowiak D. Conventional or Li vasectomy: a questionnaire study. Brit. J. urol. 1997; 79 (4): 596-8.
9. Whyte J, Sarrat R, Diaz P, Whyte A, Torres A, Cisneros A. Effects of experimental Vasectomy on testicular structure: Ultrastructural study. Actas aurol Esp. 1999; 23 (4): 309 –15.
10. Altay B, Hekimgil M, Kefi A, Cikili N. Histo-pathological examination of both ipsilateral and contralateral testes with different obstructive models in pre-pubertal and adult rats. Brit J.Urol. 2001; 88 (1): 84-9.
11. Savas C, Ozogul C, Karaoz E, Bezir M. Ishaemia, whether from ligation or torsion, causes ultrastructural changes on the contralateral testis. Scand. J. Urol Nephrol. 2002; 36(4):302-6.
12. Sanchez FJ, Villaplana TL Sarti MM. Light Microscopy of the rat testicle after Vasectomy. J. Actas Urol Esp. 1996; 20(6): 403-7.
13. Shiraishi K, Takihara H, Naito K. Influences of interstitial fibrosis on spermatogenesis after vasectomy and vasostomy. J. Contraception. 2002: 65 (3): 245- 9.
14. Shen K, Yang Y, Tang X, Huang M, Shen H, Lei S, Wei Y, Zhang H. Effect of vasectomy on apoptosis in spermatogenic cells of the male rat. Asian J. Urol. 1998: 29 (4): 402-6.
15. Wen RQ, Li SQ, Wang QH, Liu MY. Morphological changes of spermatozoa in proximal vas deferens after vasectomy. Asian J. Androl. 1999; 1 (1-2): 61-5.
16. Mcdonald SW. Cellular response to vasectomy. Int. Rev. Cytol. 2000; 199:295-339.
17. Andonian S, Jarvi K, Zini A, Hermo L. Ultrastructural features of the vas deferens from patients undergoing vasectomy and vasectomy reversal. J. Androl. 2001; 23 (5): 691-701.
18. Chevhal MJ, Doshi R, Kidd CF, Wilkelmann T, Chevhal V. Anti-sperm auto-antibody response after unilateral vas deferens ligation in rats: when does it develop? J Androl. 2002; 23 (5): 669-73.
19. Flickinger CJ, Howards SS, Herr JC. Effects of vasectomy on the *Epididymis*. Microsc. Rev. Tech. 1995; 30(1):82-100.
20. Platz EA, Yeole BB, Cho E, Jussawalla DJ, Giovannucci E, Ascherio A. Vasectomy and prostate cancer: a case control study in India. Int. J. Epidemiol. 1997; 26 (5) : 933-8.
21. Dennis LK, Dawson DV, MA. Resnick. Vasectomy and the risk of prostate cancer: a meta-analysis examining vasectomy status, age at vasectomy, and time since vasectomy, J. Prostate cancer. Prostatic disease. 2002; 5(3): 193-203.
22. Zhu K, Stanford JL, Daling JR, Mcknight B, Stergachis A, Brawer MK, Weiss NS. Vasectomy and prostate cancer: a case control study in a health maintenance organisation. Am. J. Epidemiol. 1999; 144(8):717-22.
23. Lesko SM, Louik C, Vezina R, Rosenberg L, Shapiro S. Vasectomy and prostate cancer. J. Urol. 1999; 161(6):1848-52.
24. Sarrat R, Whyte J, Torres A, Lostale F, Diaz MP. Experimental vasectomy and testicular structure. J. Histol-Histopathol. 1996; 11(1):1-6.
25. Kubota Y, Sasaki S, Kubota H, Tatsura H, John K. A study on the mechanisms of spermatogenic damage after vasectomy in rats. Nippon Hingoyika Gakkai Zasshi. 2001; 92(1): 13-22.
26. Koksal IT, Usta M, Orhan I, Abbasoglu S, Kadioglu A. Potential role of reactive oxygen species on testicular pathology associated with infertility. Asian J. Androl. 2003; 5 (2):95-9.
27. Cerasaro TS, Nachtsheim DA, Otero F, Parsons CL. The effect of testicular torsion on contralateral testis and the production of anti-sperm antibodies in rabbits. J. Urol. 1984; 132(3) 577-9.
28. Dwyer NT, Grant JE. Vasectomy and its reversal. J sex. Reprod. Med. 2002; 2(4) : 144-150
29. Edward K, Daniel H, Lipschultz L. Vasectomy reversal: new techniques and role in the era of intracytplasmic sperm injection. Canadian J. Urology. 2006; 13(1): 22-27.
30. Malaka SLO. Pers Comm. Lagos 2005; Department of Zoology of University of Lagos.
31. Buege JA, Aust SD. Microsomal lipid peroxidation in: Fleischer S, Packer L. Eds. Methods in Enzymology LII. 1978; 302-330. New york Academic press.
32. Gundersen HJG, Jensen EB. The efficiency of systematic sampling in stereology and its predictions. *J. Microsc.,* 1987; 147: 229-263.
33. Nistal M, Rierstra ML, Galmes-Belmonte I, Paniagua R: Testicular biopsy in patients with obstructive azoospermia . Am J Surg Pathol. 1999; 23: 1546-54.
34. Flickinger CJ, Baran ML, Howard SS, Herr JC. Degeneration of the seminiferous epithelium following *epididymal* obstruction in pre-pubertal rats. Anat Rec. 1999; 254: 76-86.
35. West DA, Chehval MJ, Winkelman T, Martin SA. Effect of vasovasotomy on contralateral testicular damage associated with unilateral vasectomy in mature and immature Lewis rats. Fertil Steril. 2000; 73: 238-41.
36. Cerasaro TS, Nachtsheim DA, Otero F, Parsons CL. The effect of testicular torsion on contra-lateral testis and the production of anti-sperm anti-bodies in rabbits. J. Urol. 1984; 132(3) 577-9.
37. Whyte J, Cisneros AI, Rubio E, Whyte A, Mazo R, Torres A, Sarrat A. Morphometric study of testis of Wistar Rat after open-ended vasectomy. Clin Anat. 2002; 15:335-339.

38. Richardson LL, Kleiman HK, Dym M. Altered basement membrane synthesis in the testis after tissue injury. J Androl. 1998; 19: 145- 155.
39. Aitken H, KumarakuruS, Reid O, Milne EW, Bennett NK, Mcdonald SW. Degenerated tubules in the guinea pig testis after long-term vasectomy or sham operation. Clin Anat. 2000; 13: 6-10.
40. XJoshi KR, Ramdeo IN, Sachdev SB, Bharadwaj TP. Effects of vasectomy on testes. Int Surg. 1972; 57: 711-713.