An evaluation of the deleterious effect of unilateral cryptorchidism on the contralateral normally descended testis

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Human and experimental animal models have been extensively used to elucidate the detrimental effect of unilateral cryptorchidism on ipsilateral testicular morphology and function. Uncertainty however still exists on the effect of unilateral cryptorchidism on contralateral testicular function. This study investigated the effect of left unilateral cryptorchidism on right normally descended testicular function. Forty immature rats were divided into two groups (group A, 30 rats and group B, 10 rats). Group A rats were rendered experimentally cryptorchid by anchoring the upper pole of the testis to the posterior abdominal wall. Group B rats were sham operated to serve as control. 56 days after cryptorchidism induction, bilateral testicular weight, bilateral testicular volume, bilateral caudal epididymal sperm characteristics, bilateral testicular histo-morphometry, fertility rate in vivo and serum hormone levels were all tested. Most of the above parameters were significantly lower (P<0.05) in group A (Right and Left) compared to group B. The concentrations of serum luteinizing hormone (LH) and follicle stimulating hormone (FSH) were however significantly higher (P<0.05) in cryptorchid group compared to the control. The current results indicate a bilateral impairment of germ, Sertoli and Leydig cells function and histology with unilateral cryptorchidism.

Key words: Cryptorchidism, testis infertility, rats.

INTRODUCTION

Male infertility is both a private and social problem. Cryptorchidism is a well known clinical condition associated with male infertility (Alpet and Klein, 1983; David et al., 1992; Seppo et al., 1996). The word cryptorchid literally means a testis which is hidden from view and cryptorchidism results from failure of the testis to descend into the scrotum. With an incidence of between 2 - 6 % in newborns, it is the most common disorder of sexual development at full-term birth (Ono and Sofikitis, 1997).

With the exception of elephants and whales, most mammals have a scrotum, and the scrotal temperature is always lower than that of the abdomen (Nelson, 1951; Yavetz et al., 1992). The difference of a few degrees between body and scrotum is believed to maintain optimal environment for testis function, and it is known that surgical induction of cryptorchidism in experimental animals causes disruption of spermatogenesis leading to infertility (Seppo et al., 1996). Cryptorchidism in humans can be attributable to genetic (trisomy), hormonal (defect in androgen action), and anatomical (gubernacula deformity) factors (Marshall, 1982). However, irrespective of the cause, a common defect in spermatogenesis is always detected. It is well known that the function of the cryptorchid testis is impaired by a temperature rise in unilateral cryptorchidism. The effect of unilateral cryptorchidism on ipsilateral testicular function has been well studied in human and experimental animals (Rager et al., 1975; Keel et al., 1980; Jegou et al., 1983; Saalu et al., 2006). However the effect of unilateral cryptorchidism on contralateral testicular function has not been as extensively studied. Indeed the magnitude and extent of this so called “sympathetic” testicular damage re
remains unclear.

The aim of the present study therefore is to evaluate the effect of left artificial cryptorchidism on the right testicular and epididymal functions and morphology.

MATERIALS AND METHODS

Animals

4 weeks old immature male Sprague-Dawley rats weighing 90 – 120 g were used for the study. The animals were housed in wire mesh cages under standard environmental conditions with the provision of 12 h light and 12 h darkness. Rat cubes (Pfizer feeds Nigeria Limited, Lagos, Nigeria) and water were provided and libitum.

Experimental protocol

Forty male rats were weighed and divided randomly into two groups. Group A (30 rats) served as the experimental group in which the rats were rendered unilateral cryptorchid. To induce cryptorchidism, the rats were anaesthetized with intra-abdominal injection of 7 mg kg\(^{-1}\) body weight ketamine hydrochloride.

A 2 cm left paramedical incision was made through the skin, beginning 3 – 4 cm caudal to the prepuce and extended cranially. The left gubernaculum was cut and the testis displaced into the abdomen. The upper pole of the left testis through its tunica albuginea was fixed with a 4 - 0 Nylon suture to the left psoas muscle in the posterior abdominal wall (Ono and Sofikitis, 1997). Group B (10 rats) which served as control were sham operated. Through a left paramedical incision, there was placement of the left testis into the abdomen and replacement of the testis into its normal position. 56 days after cryptorchidism induction, all the rats were processed for the assessment of their fertility potential in vivo.

Thereafter all the rats were sacrificed by decapitation. Testicular weight and volume, epididymal sperm characteristics, serum hormone levels and testicular histology were evaluated.

Fertility potential in vivo

Two fertile female Sprague-Dawley rats in the first hours of estrus as determined by vaginal smear examination were placed in a single cage with each male rat. 2 h later, the female rats were checked after mating to detect spermatozoa in their vagina by microscopic examination of the vaginal fluid. Females in which spermatozoa were detected were then checked 3 times daily from day 21 for parturition (day of mating taken as day 1). A male rat was considered fertile if its mating resulted in at least one pregnancy.

Organ weight and volume estimation

The testes were excised, dissected free of surrounding tissue, their weight determined and volume measured by water displacement method.

Sperm characteristics:

The testes from each rat were carefully exposed and removed. They were trimmed free of the epididymides and adjoining tissues. From each separated epididymis, the cauda part was removed and placed in a beaker containing 1 mL physiological saline solution. Each section was quickly macerated with a pair of sharp scissors and left for a few minutes to liberate its spermatozoa into the saline solution. Sperm motility, concentration and progressive motility were determined as earlier described (Raji et al., 2005, 2006). Semen drops were placed on the slide and two drops of warm 2.9% sodium citrate were added. The slide was covered with a cover slip and examined under the microscope using X40 objective for sperm motility. Sperm count was done under the microscope using improved Neubauer haemocytometer.

Serum biochemistry

The serum levels of LH, FSH and testosterone were measured by validated radioimmunoassay (RIA) technique (Raji et al., 1997). The respective intra and inter assay variations were 10.4 and 10.23% for testosterone; 10.30 and 9.80% for LH and 9.60 and 10.10% for FSH. The RIA reagents used were those of the World Health Organization Matched Reagent Program, supplied by NIADDK – NIH (USA). All solvents and mineral acids used were of the analytical grade.

Histological analysis

The organs were cut in slabs of about 0.5 cm thick and fixed in Bouin’s fluid for a day after which it was transferred to 70% alcohol for dehydration. The tissues were passed through 90% alcohol and chloroform for different durations before they were transferred into two changes of molten paraffin wax for 20 min each in an oven at 57°C. Prior to embedding, it was ensured that the sections to be cut by the microtome were oriented perpendicular to the long axis of the testis. Serial sections of 5 µm thick were obtained from a solid block of tissue and were stained with haematoxylin and eosin stains, after which they were passed through a mixture of equal concentration of xylene and alcohol. Following clearance in xylene, the tissues were oven – dried. Light microscopy was used for the evaluations.

Morphometric analysis

For each testis five sections from the polar and equatorial regions were sampled and an unbiased numerical estimation of the mean seminiferous tubular diameter (STD) was done using a systemic random scheme (Gundersen, 1987). The mean STD was derived by taking the average of two diameters \(d_1\) and \(d_2\). \(d_1\) and \(d_2\) are taken only when \(d_1/d_2 ≥ 0.85\).

Statistical analysis

Data are expressed as mean ± standard error of the mean (M ± SEM). The significance of difference was at \(p< 0.05\). Statistical analysis was performed using the student t-test and ANOVA.

RESULTS

Fertility rate in vivo

The effects on infertility are shown in Table 2. The proportion of fertile rats in the cryptorchid group was significantly lower (\(P<0.05\)) than in the control group.

Weight and volumes of testes

Table 1 shows that left testicular weight and volume were
Table 1. Testicular weight (g) and volume (mL), and seminiferous tubules (ST) diameter (µm).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cryptorchid (n = 30)</th>
<th>Control (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td>Weight</td>
<td>0.196 ± 0.12*</td>
<td>0.86 ± 0.10*</td>
</tr>
<tr>
<td>Volume</td>
<td>0.180 ± 0.14*</td>
<td>0.87 ± 0.12*</td>
</tr>
<tr>
<td>ST diameter</td>
<td>120.43 ± 2.36*</td>
<td>146 ± 1.23*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM.
* P<0.05 compared to control group.

Table 2. Sperm characteristics and fertility.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cryptorchid (n = 30)</th>
<th>Control (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td>Concentration (x 10^6 mL^-1)</td>
<td>1.25 ± 6.1*</td>
<td>15.3 ± 0.7*</td>
</tr>
<tr>
<td>Sperm motility (%)</td>
<td>7.5 ± 2.1*</td>
<td>30.5 ± 9.0*</td>
</tr>
<tr>
<td>Progressivity</td>
<td>b1</td>
<td>b1</td>
</tr>
<tr>
<td>Fertile</td>
<td>4 [13.3]*</td>
<td>9 [90]</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SEM.
* P < 0.05 compared with the control group.
[ ] percentage.

a1 = Rapid linear progressive motility; b1 = sluggish linear or non-linear motility.

Table 3. Serum hormone levels (ng mL^-1).

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Cryptorchid (n = 30)</th>
<th>Control (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone</td>
<td>1.05 ± 0.40*</td>
<td>2.50 ± 0.45</td>
</tr>
<tr>
<td>FSH</td>
<td>0.5 ± 0.31*</td>
<td>0.25 ± 0.15</td>
</tr>
<tr>
<td>LH</td>
<td>16.43 ± 6.23*</td>
<td>8.94 ± 2.36</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM.
* P < 0.05 compared with the control group.

significantly lower (P<0.05) than the right testicular weight and volume in cryptorchid rats. The right testicular weights and volumes in cryptorchid rats were also significantly lower (P<0.05) compared to the testicular weights and volumes of the control group.

Epididymal sperm characteristics

As shown in Table 2, the sperm content and percentage motility were significantly lower (p<0.05) in the left epididymides than the right epididymides of the cryptorchid rats. However the caudal epididymal sperm concentration and percentage sperm motility were significantly lower (p<0.05) bilaterally in the cryptorchid group compared to the control rats. The sperm cells from both caudal epididymides of the cryptorchid rats showed sluggish non linear movement while those from the control group demonstrated rapid linear motility.

Fertility potential in vivo

As summarized in Table 2, the proportion of fertile rats in the cryptorchid group was significantly lower (p<0.05) than the control group.

Serum hormone levels

The peripheral serum basal testosterone levels of the cryptorchid rats were significantly lower p<0.05 than the control group. In contrast, the serum FSH and LH levels of the cryptorchid group were significantly higher (p<0.05) compared to the control rats (Table 3).

Histo-morphometry

Light microscopy was used for evaluation of testicular histology as shown in Figures 1–3. The seminiferous tubules of the control rats were completely and fully differentiated. Spermatozoa are shown in some of the tubules. However in the cryptorchid group, the testis showed atrophy of the seminiferous tubules, degeneration of the germinal epithelium and absence of late stage germ cells. This was more marked in the left cryptorchid testis than in the right unmanipulated testis. The mean seminiferous tubular diameters of bilateral testes in cryptorchid rats were significantly lower (P < 0.05) than those of the control group. In the cryptorchid group, the left (abdominal) testes mean seminiferous diameter was significantly lower (P < 0.05) compared to that of the right (scrotal) testes (Table 1).

DISCUSSION

Undescent of the testis results in deterioration of the
affected testis. The generally accepted explanation for the deterioration is the higher temperature in the abdominal cavity (Chowdhury et al., 1994). This view is supported by the tubular changes following prolonged febrile illness in man and other experimental studies (Wolley, 1979). The descent of only one testis is also frequently accompanied by infertility (Huff et al., 2001). Indeed in congenital unilateral cryptorchid rats, the fertility rate is 0% compared with 100% in control rats (Patkowski et al., 1992). In the present study a model of cryptorchidism was created in rats at 4 weeks of age. Our study demonstrated that unilateral cryptorchidism induced in the immature rats before sexual maturation resulted in a significant bilateral impairment on all testicular functions, including spermatogenesis, Sertoli cell and Leydig cell functions.

The significant bilateral decrease in the mean testicular weight and volume in the cryptorchid rats indicates bilateral testicular dysfunction involving both spermatogenesis and steroidegenesis. This is because as shown by Takihara et al. (1987), testicular size has a positive correlation with testicular function. Previous experimental studies have suggested that mean seminiferous tubular diameter is a more sensitive early indicator of contra-late-
r al testicular deterioration (Karaguzhevi et al., 1995; Zhang et al., 2002). Our study with data showing signi-ficant reduction in the mean seminiferous tubular diameters in cryptorchid rats therefore corroborates these findings.

A detrimental effect of unilateral testicular function is additionally indicated by the significant reduction of the quantitative and qualitative sperm parameters bilaterally in animals with left cryptorchidism. Logical fallout of these poor sperm characteristics in both the left and right testis of the cryptorchid group is the significant reduction in the fertility potential in vivo observed in this group.

An impaired secretory function of Leydig cells bilaterally in cryptorchid rats was demonstrated in our study as shown by significant reduction of testosterone level associated with elevated LH levels. The elevated LH levels indicate a negative feedback resulting from Leydig cells failure to produce optimal testosterone levels (Jegou et al., 1984). As suggested by Karpe et al. (1981), the elevated FSH levels in the manipulated rats could mean Sertoli cells functional deficiency. This is because Sertoli cells produce androgen binding proteins (ABP) under the control of FSH which is elevated by a feed back mechanism in Sertoli cellular derangement. In contrast to these findings however Shaked and Sheshel et al. (2001) demonstrated a normal steriodogenic function of the somatic cells (Leydig and Sertoli) when testis is exposed to core body temperature. Similar data was also obtained from our previous study (Saalu et al., 2006) using adult animals. The variations could therefore be due to the differences in the timing of cryptorchidism induction.

Although the effect of unilateral cryptorchidism on ipsilateral testicular function may be attributable to the increase in ipsilateral testicular temperature, there is much controversy concerning the mechanisms by which unilateral cryptorchidism produces contralateral testicular toxicity. Ono and Sofikitis (1997) have demonstrated an increase in contralateral testicular blood flow and temperature in animals with unilateral cryptorchidism. Contralateral testicular deterioration may therefore result from a reflex mechanism probably as in conditions such as consensual ophthalmic reflex and reflex anuria.

In conclusion, our study demonstrated a detrimental effect of left cryptorchidism on the right testicular function. These findings if found to be progressive will support the clamour for early orchidopexy in conditions of unilateral cryptorchidism to protect both the ipsilateral and contralateral testes.

REFERENCES


