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Comparative studies of fertility and histologic development of contralateral scrotal testes in two rat models of unilateral cryptorchidism

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Abstract Fertility and the development of the contralateral scrotal testis in patients with unilateral cryptorchidism (UCO) remain controversial. This study investigated these controversies in two different UCO rat models using 43 Wistar King A rats. The animals were divided into three groups: I: an endocrinologic model of UCO was obtained by injecting pregnant dams with flutamide 100 mg/kg per day on days 15–17 of gestation (n = 12); II (n = 21): a mechanical model of UCO was obtained by extra-abdominal fixation of the gubernaculum in the neonatal period, III (n = 10): non-treated rats were used as controls. At the age of 90 days, 5 rats from each group were segregated into individual cages and housed with two virgin adult females for 2 weeks. The occurrence of pregnancy and litter sizes were counted in order to study fertility. All the animals were then weighed and killed. The occurrence of testicular descent, growth of the external genitalia, and epididymal development were examined. Morphologic and histologic evaluations were performed in the cryptorchid and contralateral testes. In the endocrinologic model (group I) the 10 female rats failed to show any offspring (0%), while in the mechanical model (group II) 9 out of 10 rats had offspring (90%, P < 0.001); 10 out of 10 control rats showed offspring. All of the rats in groups I and II had UCO, and the descended testes were located in the superficial inguinal position, while the contralateral and control testes descended into the scrotum. Hypospadias and a small epididymis were frequently noted in the flutamide-treated rats. Testicular weight, seminiferous tubular diameter, and spermatogenesis were all significantly reduced in the descended testes (UDT) compared to the contralateral and control testes. Moreover, the development of the contralateral testis was inhibited in group I compared to groups II and III. Our observations showed that short-term exposure to flutamide in utero induced significantly reduced fertility and degenerated contralateral scrotal testes in UCO rats compared to mechanically-induced UCO rats by early adulthood. It is suggested that fertility potential and testicular development in unilateral UDT may be partially due to the factors that induce testicular maldescend, especially in cases due to intrauterine hormonal abnormalities. These cases may show inhibited fertility and testicular development even after orchiopexy.

Key words Fertility · Histologic development · Unilateral cryptorchidism

Introduction

Although cryptorchidism is a clinical condition that has been recognized for thousands of years, many aspects of this disease process remain poorly understood. Among the numerous reasons, is the inability to study the disease scientifically in humans. It has been agreed that a close association exists between testicular maldescend and infertility, and consequently, there is a high incidence of infertility in bilateral cryptorchidism [7, 16], yet testicular development and fertility remain controversial in cases of unilateral cryptorchidism (UCO). Many authors have suggested in studies of UCO in both humans and animals that fertility is inhibited compared to normal controls [2, 4, 7, 16, 19]. On the other hand, some investigators have suggested no significant inhibition of fertility in UCO [1, 3, 11, 18]. Furthermore, there has been disagreement about the development of the contralateral scrotal testis in UCO.

Several publications have described degeneration of the contralateral testis in UCO [2, 4, 15]. This concept has been supported by various authors using experimental UCO models [14, 17, 19, 22]. Others have suggested that the contralateral scrotal testis is not affected in UCO [6, 10]. In a previous study, we had shown significantly inhibited development of the contralateral
testis in an endocrinologic model of UCO, although there was not any significant developmental inhibition of the contralateral testes in the mechanical model of UCO induced by neonatal gubernacular fixation [24]. This study aimed to investigate fertility in both the endocrinologic and mechanical models of UCO in order to investigate whether the causes of unilateral undescended testis (UDT) may affect the fertility and development of the contralateral scrotal testis.

Materials and methods

Wistar King A rats obtained from a colony maintained at Kyushu University (Fukuoka, Japan) were used in the present studies, and the pure antiandrogen flutamide (Nihon Kayaku, Tokyo) was also used. The rats were divided into three groups: in group I (12 rats) an endocrinologic model of UCO was obtained by injecting the pregnant dams with flutamide 100 mg/kg per day on days 15–17 of gestation. In group II (21 rats) a mechanical model was obtained by extra-abdominal fixation of the gubernaculum in the neonatal period [21]. In group III (10 rats) non-treated rats were used as controls.

The pups were raised by their mothers in an air-conditioned (23 ± 2 °C) and light-controlled (12 h light/12 h dark) environment with rat chow and water ad libitum. At 30 days of age, all pups were weaned. At the age of 90 days, the occurrence of UCO was confirmed in groups I and II and 3 rats from each group were segregated into individual cages and housed with two virgin adult females for 2 weeks. The occurrence of pregnancy and litter sizes were counted in order to determine fertility. All the animals were then weighed and killed at the age of 115–120 days. The position of the UDT growth of the external genitalia, and epididymal development were examined, and then all testes were weighed and fixed in 10% formalin and embedded in paraffin. The specimens were sectioned at 5 μm thickness and stained with hematoxylin and eosin.

The histologic examination was done blindly by a single observer. The seminiferous tubular diameter (STD) was measured using a micrometer eyepiece (10x) and mean STD was calculated by averaging the diameter of 50 round, randomly-selected seminiferous tubules in each tissue section. The assessment of the degree of testicular development was performed using the Johnsen score (scale 1–10) [5]. The mean value of the Johnsen score for 50 seminiferous tubules in each section was calculated for every testis. Statistical analysis of the data was performed using the chi-square test and Student’s t-test. P-values below 0.05 were considered statistically significant.

Results

In group I (5 endocrinologically-induced UCO rats) the 10 female rats failed to show any offspring (pregnancy rate 0%). In group II (5 mechanically-induced UCO rats) 9 out of 10 females showed offspring (pregnancy rate 90%, P < 0.001). In group III (5 normal male rats) the males were able to impregnate all 10 females (pregnancy rate 100%, Table 1).

At 90 days of age, hypospadias and a small, rigid scrotum were frequently observed in the endocrinologic model. Epididymal abnormalities (nonunion of the caudal epididymis) were also found in 4 out of 12 descended testes in group I. However, epididymal abnormalities were not noted in either the mechanical model or the control rats. Unilateral UDTs were all located in the superficial inguinal position in groups I (n = 12) and II (n = 21). By contrast, all control rats showed normally descended testes (n = 10).

The histologic appearance of UDTs showed a reduced STD, no spermatozoa, and inhibited development of germ cells compared to the contralateral and/or control testes in groups I and II (Fig. 1). However, there were no significant differences in testicular weight, STD, and Johnsen score of the cryptorchid testes between groups I and II. In contrast, in the contralateral scrotal testes testicular weight, STD, and Johnsen score were all significantly reduced in group I compared with group II and the controls.

Testicular weights of the contralateral testes were 0.19 ± 0.02 g/100 g body weight in groups I, 0.39 ± 0.01 in group II, and 0.38 ± 0.02 in group III (P < 0.001; group I vs groups II and III). The STD of the contralateral testis was 0.22 ± 0.01 mm in group I, 0.28 ± 0.005 mm in group II, and 0.29 ± 0.005 mm in group III (P < 0.001; group I vs groups II and III). Furthermore, the Johnsen score indicated significantly inhibited spermatogenesis in group I (6.96 ± 0.63) compared with group II (9.62 ± 0.06) and the controls (9.73 ± 0.06, P < 0.01; group I vs groups II and III). There was no significant differences in testicular weight, STD, and Johnsen score between the contralateral testes in group II and the control testes in group III.

Discussion

The most important goal in the treatment of cryptorchidism is to preserve the potential for fertility. This is a highly debatable topic; there have been many opinions regarding the likelihood of fertility in UCO. Kogan [7] reported a fertility rate of 45% in the UCO rat model of intra-abdominally fixed testes. Ratief [16] suggested complete infertility in patients with unilateral UDT, especially when operated upon at a late age. Other authors have reported fertility to be within the normal average range of 67% to 87% after orchiopexy [1, 3, 11, 18]. According to Lipshultz et al. [8], successful fertility after orchiopexy in UCO may be related to the growth and differential development of the contralateral scrotal testis.

In Wistar King A rats, males are highly fertile and females have multiple pregnancies. Thus, mating studies involving these rats should detect subtle changes in fertility. In our study, the contradictory results concerning the fertility and development of the contralateral testes were obtained from two different endocrinologic and

| Table 1 Fertility among female rats at 90–115 days |
|-------------------|-------------------|-------------------|-------------------|-------------------|
| Rats | Group I (10 females) | Group II (10 females) | Group III (10 females) | P-value |
| Pregnancy rate | 0%* | 90%* | 100% | *P < 0.01 |
