EFFECT OF HUMAN CHORIONIC GONADOTROPIN BEFORE SPERMATIC VESSEL LIGATION IN THE PREPUBERTAL RAT TESTIS

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ABSTRACT

After single stage Fowler-Stephens orchiopexy testicular atrophy is common. Previous experimental study in the scrotal testis of the adult rat has shown that ligation of the internal spermatic artery often causes focal testicular infarction or atrophy and exogenous human chorionic gonadotropin increases testicular blood flow. We questioned whether division of the internal spermatic artery in the undescended rat testis would also cause testicular injury and hypothesized that these changes might be prevented by administering human chorionic gonadotropin before vessel ligation. Two groups of 20-day-old Sprague-Dawley male rats were subjected to unilateral internal spermatic artery ligation. Group 1 (control) received no human chorionic gonadotropin, while group 2 received 10 IU human chorionic gonadotropin daily from days 3 to 17 of life. At 3 months testicular blood flow was quantitated using $^{141}$cerium radioactive microspheres. The results indicated that blood flow on the operated side was lower than on the nonoperated side but the change was not statistically significant. Human chorionic gonadotropin had no effect on blood flow to the operated testes. Gross testicular weights of operated versus nonoperated gonads were similar in rats that did and did not receive human chorionic gonadotropin. Pathological evaluation revealed normal histology in all testes. These results do not support the use of human chorionic gonadotropin preoperatively to stimulate collateral testicular blood flow.

KEY WORDS: testis; gonadotropins, chorionic; rats, inbred strains

Approximately 1% of male infants have an undescended testis and undergo orchiopexy. As many as 20% of undescended testes are nonpalpable of which many are intra-abdominal. In many boys with an intra-abdominal testis the orchiopexy (Fowler-Stephens abdominal) to allow placement of the testis in the scrotum. After Fowler-Stephens orchiopexy viability of the testis depends primarily on collateral circulation through the deferential artery and testicular atrophy occurs in approximately 20% of patients. The observed postoperative changes in these gonads have been attributed to an inadequate or injured collateral blood supply. Clinical success with 2-stage Fowler-Stephens orchiopexy is superior to the 1-stage procedure, presumably because of disruption or vasospasm of delicate collateral vessels in the 1-stage procedure.

In the postpubertal rat with scrotal testes division of the spermatic vessels has been reported to cause varying degrees of testicular atrophy. In this animal model ligation of the spermatic vessels initially causes a significant decrease in testicular blood flow but it ultimately returns to normal. Human chorionic gonadotropin has been reported to cause a significant increase in testicular blood flow in the mature rat. We questioned whether ligation of the testicular artery of an undescended testis in the prepubertal rat would cause testicular injury and whether any changes could be prevented by administering human chorionic gonadotropin before operative manipulation.

MATERIALS AND METHODS

A total of 20, 3-day-old Sprague Dawley male rats was housed 10 animals per cage with a lactating female rat. The female rat in each cage had free access to food and water. The juvenile male rats were randomly divided into 2 groups of 10 animals each. Group 1 served as a control while group 2 received daily subcutaneous injections of 10 IU human chorionic gonadotropin for 14 days beginning on day 3 of life. At age 20 days the male rats underwent right internal spermatic vessel ligation under inhalational ether anesthesia. A midline transabdominal incision was made and the right colon was mobilized. Using a dissecting microscope the right ureter was identified at the right renal hilum, and the right internal spermatic vessels were identified and ligated with a 6-zero silk ligature (operated testis). The right colon was then returned to its normal anatomical position. The left ureter was also exposed in each rat (nonoperated testis). The animals were allowed to wean and had free access to food and water during the next 2 months. Eventually they were separated and housed 2 animals per cage.

At age 3 months the experimental animals were anesthetized with 50 mg./kg. sodium pentanembutol intraperitoneally. The left inguinal fossa and hemithorax were shaved and prepared with povidone-iodine scrub. A left inguinal incision was made and the left femoral artery was cannulated with a 0.58 mm. internal diameter polyethylene catheter, which was secured with a 6-zero silk ligature (operated testis). The right ureter was also exposed in each rat (nonoperated testis). The animals were allowed to wean and had free access to food and water during the next 2 months. Eventually they were separated and housed 2 animals per cage.

A Harvard pump set at a constant withdrawal rate of 0.3 cc per minute. Simultaneously, the Harvard pump was turned on and left thoracotomy was performed. Following vortex mixing of $^{141}$cerium ($^{141}$Ce) microspheres the appropriate volume containing 7 $\mu$Ci. was drawn up into a 0.5 cc syringe and 23 gauge needle. The muscles of the left chest were exposed and dissected off the rib cage, affording access to the ribs and intercostal muscles. The femoral arterial line was connected to a Harvard pump set at a constant withdrawal rate of 0.3 cc per minute. Simultaneously, the Harvard pump was turned on and left thoracotomy was performed. Following vortex mixing of $^{141}$cerium ($^{141}$Ce) microspheres the appropriate volume containing 7 $\mu$Ci. was drawn up into a 0.5 cc syringe and 27 gauge needle. The solution was then injected into the left atrium (10 rats) or left ventricle (10 rats). Blood from the syringe in the Harvard Pump was transferred to a carrier in which radioactivity could be counted by a gamma well counter. The syringes were successively washed with a saline solution that was counted until the level of radioactivity in the washing was equal to a previously determined background level of activity.

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The left and right testicles were removed from each animal via a transcrotal approach and the perigonadal tissues, including the epididymis, were removed, leaving only the tunica albuginea and its contents. The gonads were then placed into test tubes of known weight and gonadal radioactivity was recorded, as was the wet weight of each gonad. Based on the measured radioactivity from the blood and testicles as well as the weight of the testicles, quantitation of testicular blood flow was calculated according to the following formulas:

\[
\text{Total blood counts corrected} = \frac{\text{Counts per ml. per minute}}{\text{Weight of withdrawn volume of blood}} \times \text{Time of blood withdrawal,}
\]

\[
\text{Flow (ml. per minute)} = \frac{\text{Tissue counts}}{\text{Counts per ml. per minute}} \times \frac{\text{Flow}}{\text{Wet tissue weight}}.
\]

Mean values for testicular blood flow in ml. per minute were calculated for the operated and nonoperated gonads in human chorionic gonadotropin treated and control animals. In addition, testicular blood flow in ml/gm. testis was calculated for all testes. Statistical analysis was performed using the 2-tailed Student t test. The testes were fixed in Bode's solution, embedded in paraffin, and stained with hematoxylin and eosin. At least 4 sections from each testis were examined by a single pathologist without knowledge of the treatment group.

RESULTS

All animals survived the operative procedure performed at age 20 days. At 3 months technical problems precluded satisfactory assessment of testicular blood flow in 3 rats undergoing atrial injection of $^{141}$Ce (1 in the human chorionic gonadotropin and 2 in the no human chorionic gonadotropin group) and 1 rat undergoing ventricular injection (no human chorionic gonadotropin group). The results of blood flow studies are shown in the table. Testicular blood flow was lower in gonads subjected to spermatic vessel ligation compared to nonoperated testes but this difference was not statistically significant ($p = 0.11$ in the no human chorionic gonadotropin and $p = 0.22$ in the human chorionic gonadotropin group). There was no demonstrable improvement in testicular blood flow in testes that had been exposed to human chorionic gonadotropin compared to the control.

Testicular wet weights were similar in all animals. All histological sections examined had normal findings except that the microspheres could be seen in the testicular microcirculation. There were no discernible differences between any of the slides examined. Specifically, the parenchyma-to-stroma ratio was normal. There was no evidence of edema, fibrosis, Leydig cell hyperplasia or inflammation in any of the sections. There was also no difference in the amount of spermatogenesis and presence of Sertoli cells. No infarcts or areas of necrosis were noted in any of the sections.

**DISCUSSION**

Approximately 8% of cryptorchid testes are intra-abdominal. Division of the internal spermatic vessels is often necessary to gain sufficient mobilization of the gonad to allow scrotal fixation. After Fowler-Stephens orchiopexy the testis must survive on collateral flow through the deferential artery. Previously, Pascual et al demonstrated that in adult rats with scrotal testes division of the internal spermatic artery caused an immediate 80% decrease in testicular blood flow but flow returned to pre-treatment levels by 30 days after ligation. Geesaman et al showed that the administration of human chorionic gonadotropin to adult rats every other day caused testicular blood flow nearly to double immediately following the final injection.

We theorized that division of the internal spermatic artery in the prepubertal undescended rat testis would result in testicular injury with concomitant decrease in blood flow to the testis. We also hypothesized that administering human chorionic gonadotropin for 2 weeks before ligation might protect the testis from ischemic damage by increasing collateral blood flow. Our results indicated that at age 20 days (the age of operative manipulation) division of the testicular artery caused a decrease in testicular blood flow but the change was not statistically significant. The administration of human chorionic gonadotropin before internal spermatic artery ligation had no demonstrable effect on the parameters measured. Furthermore, the testes had normal weight and morphology when examined 2 months later. These findings are different from those of others. For example, Ortolano and Nasrallah reported that spermatic artery ligation in adult rats resulted in a 34% decrease in testicular size and in 35-day-old (immature) rats it resulted in a 66% decrease in testicular size. Furthermore, 80% of the testes manifested various elements of infarction. Kelly et al reported that spermatic vessel ligation resulted in testicular sizes that were 6 to 21% of controls. Pascual et al found that, although the testicular weights of adult rats were not affected by spermatic vessel ligation, 83% had a variable number of necrotic tubules. Salman and Fonkalsrud reported similar histological changes in 3-month-old Wistar rats subjected to spermatic vessel ligation. Because the abnormal testicular changes in these studies were more significant in immature than mature rats, we predicted that at age 20 days (just before testicular descent) most testes subjected to spermatic vessel ligation would fail to develop normally. Furthermore, we thought that studying the effect of internal spermatic vein ligation before spontaneous testicular descent would be more relevant to the clinical application of Fowler-Stephens orchiopexy in the human, rather than investigating the effects on descended testes, as reported by others. However, all testes were normal in size and had normal histology. Pascual et al performed spermatic vessel ligation in 10-day-old rats and found that testicular blood flow at 60 days was 82% of controls ($p < 0.02$), which is a result similar to ours with the exception that our study did not achieve statistical significance.

The difference between our histological results and those of others is difficult to explain. Kelly et al performed spermatic vessel ligation in the inguinal canal. The testes in their

<table>
<thead>
<tr>
<th>Operated Testes</th>
<th>Nonoperated Testes</th>
</tr>
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<tbody>
<tr>
<td><strong>Human Chorionic Gonadotropin (9 rats)</strong></td>
<td><strong>No Human Chorionic Gonadotropin (7 rats)</strong></td>
</tr>
<tr>
<td>Mean blood flow (ml/min)</td>
<td>$0.0968 \pm 0.023$</td>
</tr>
<tr>
<td>Mean blood flow (ml/gm)</td>
<td>$0.049 \pm 0.012$</td>
</tr>
<tr>
<td>Testicular wt. (gm.)</td>
<td>$1.927 \pm 0.068$</td>
</tr>
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$P$ value was not statistically significant.
study were quite small and fertility was 0%. It is conceivable that the collateral blood supply was impaired during operative manipulation. In other studies spermatic vessel ligation was performed in the retroperitoneum, using a technique similar to ours. The primary difference was the age at which ligation was performed. Our results suggest that in the prepubertal rat collateral testicular blood flow may be better developed than in older rats and, therefore, the 20-day-old rat testis is protected from infarction after interruption of the main testicular blood supply.

In developing an animal model that has potential application to the clinical use of Fowler-Stephens orchiopexy study of the immature rat testis theoretically is more applicable than that of the adult scrotal testis. The ideal model would include Fowler-Stephens orchiopexy of the undescended testis. Until such studies are performed, preoperative treatment with human chorionic gonadotropin in children with an intra-abdominal testis for the purpose of improving the outcome of single stage Fowler-Stephens orchiopexy does not seem warranted.

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REFERENCES