Short Communication

Age-dependent Decrease in Incidence of Rat Sperm Granulomas Induced by L-Cysteine

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Abstract: The pathogenesis of sperm granulomas is very complicated, and leakage of spermatozoa into extraluminal tissues is regarded as a crucial event. We previously reported that sperm granulomas were frequently induced in the epididymides of pubertal rats by excessive dose of L-cysteine (L-Cys). Here, we compared the incidence of sperm granulomas between L-Cys-treated pubertal (6-week-old) and adult (11-week-old) rats by intraperitoneally injecting daily with L-Cys (800 mg/kg body weight) for 4 weeks. L-Cys-induced sperm granulomas were seen in 6 rats of 10 L-Cys-treated pubertal rats, whereas only one rat in 40 L-Cys-treated adult rats developed a sperm granuloma, indicating age-dependent decrease in the frequency. In L-Cys-treated pubertal rats, additionally, small ducts (indicative of immaturity), dilated ducts, and interstitial edema were frequently seen, but such findings were rarely present in L-Cys-treated adult rats. The accumulation of spermatozoa in epididymal ducts was often observed both in L-Cys-treated pubertal and adult rats. These findings indicated that the administration with a large amount of L-Cys might have disturbed the luminal microenvironments, with greater influence on pubertal rats than adult rats. Particularly, the findings, that the incidences of small ducts and sperm granulomas were high in L-Cys-treated pubertal rats and low in L-Cys-treated adult rats, suggested that the prerequisite for sperm granuloma formation might be associated mainly with the delayed maturation of epididymal ducts seen exclusively in pubertal rats due to excessive dose of L-Cys.

Key words: L-cysteine, sperm granuloma, epididymis, adult rat
at 23°C ± 2°C and 55% ± 10% relative humidity, with a 12-hour light-dark cycle (lights on at 07:00) and 13–16 air changes per hour.

The L-Cys used in this study was purchased from Ajinomoto Co. (Tokyo, Japan). A 5% isotonic solution of L-Cys was prepared in distilled water and adjusted to pH 7.0 with sodium hydroxide solution. These dosing solutions were prepared immediately before use.

Forty 11-week-old adult rats were intraperitoneally injected daily with 5% L-Cys solution at the dose of 800 mg/kg body weight (16 ml/kg body weight), and 10 rats were examined each at 1, 2, 3, and 4 weeks (study weeks) after the first injection. Ten 6-week-old pubertal rats were intraperitoneally injected daily with 800 mg/kg of L-Cys, and ten 11-week-old adults served as physiological saline-treated controls; these rats were examined at study week 4 (Table 1). A preliminary study showed that the dosage of L-Cys 800 mg/kg/day was able to induce sperm granulomas in pubertal rats without death. Rats were observed daily and weighed twice weekly. All rats used were killed under ether anesthesia at each study week, and the epididymides, testes, and ventral prostate glands were removed and weighed. The weight of these organs relative to the total body weight was calculated. This study was approved by our Institutional Committee on the Care and Use of Laboratory Animals, and all procedures were performed according to institutional guidelines.

The epididymides and prostate glands were fixed in 10% neutral buffered formalin, and the testes were fixed in 12.5% formalin containing 4% acetic acid (FA solution). The tissues were embedded in paraffin, and 4-µm-thick sections were stained with hematoxylin and eosin (HE) for microscopic evaluation.

The data for body weights, and absolute and relative organ weights were compared by Student’s t-test between L-Cys-treated rats and control rats.

Immediately after injection, all L-Cys-treated pubertal and adult rats exhibited a decrease in spontaneous activity for a few hours. This symptom was gradually decreased with study week; however, the symptom seen in the pubertal rats was milder than that in adult rats, and in the pubertal rats, the symptom completely disappeared until study week 1.

During the administration period, the body weight gain in the L-Cys-treated adult rats was significantly depressed (Fig. 1, Table 1).

As shown in Table 1, a significant decrease in absolute organ weights of epididymides and prostate glands was observed in L-Cys-treated adult rats. However, the absolute organ weight of testes, as well as the relative organ weights of epididymides, testes, and prostate glands did not show significant differences between L-Cys-treated adult and control rats (Table 1).

At study week 4, sperm granulomas developed in 6 of 10 pubertal rats treated with L-Cys; the lesions were composed of a center mass of spermatozoa surrounded by many radially arranged epithelioid cells and connective tissue with a small number of lymphocytes and neutrophils in the outer layer (Fig. 2). In L-Cys-treated pubertal rats, in
addition, the segmental small ducts, dilated ducts, interstitial edema, and accumulation of spermatozoa (Fig. 3) were observed (Table 2); the anatomical location and histological characteristics of these lesions seen in the present study were in keeping with those reported previously. The term “accumulation of spermatozoa” were used for ducts occupied by spermatozoa and acidophilic inspissated materials; the lesion was observed exclusively in the caudal epididymides of L-Cys-treated rats. The small ducts were rimmed by pseudostratified epithelial cells.

On the other hand, out of 40 adult rats treated with L-Cys, only one rat at study week 4 developed sperm granuloma in the epididymides. Other findings such as dilated ducts, small ducts and interstitial edema were seen each in one adult rat at study week 3 or 4 (Table 2). The accumulation of spermatozoa at study weeks 2, 3, and 4 was
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seen in 3, 8, and 6 adult rats treated with L-Cys, respectively (Table 2).

No histological abnormalities were observed in the testes (including spermatogenesis) and prostate glands in any animals in this study.

Sperm granuloma is an inflammatory response to spermatozoa released into the interstitium, and the rupture of ducts in the epididymides is regarded as an initial lesion in the pathogenesis of sperm granuloma1–3. Previously, we showed for the first time that an excessive dose (1,000 mg/kg) of L-Cys to pubertal rats gave rise to sperm granulomas6. Dose level (800 mg/kg) used in the present study was selected, because the influences of L-Cys on clinical sign and mortality were greater in adult rats compared with those in pubertal rats. The different sensitivity in clinical signs between adult and pubertal rats in this study may be dependent on difference in the requirement for sulfur-containing amino acids (SAA) including L-Cys. It is well known that requirement for SAA is highest in infants and children7. L-Cys, although considered a non-essential amino acid, has been suggested as being essential for premature infants due to low hepatic cystathionase activity8–10.

Our previous study suggested that the development of sperm granuloma in pubertal rats might be associated with prolonged maturation of epididymal ducts resulting in obstruction of semen flow, leading to ductal rupture and the leakage of spermatozoa into interstitial tissues8. In the present study, in fact, such abnormal findings as small ducts, dilated ducts, interstitial edema, and accumulation of spermatozoa were often seen in L-Cys-treated pubertal rats, indicating the presence of immature ducts and abnormal sperm fluid. It has been considered that the ductal obstruction in pubertal rats might be caused by small ducts with pseudostratified epithelial cells and following increased viscosity of the epididymal fluid. Additionally, the small ducts (immature type) might lack contraction of the smooth muscle layer that induce sperm stasis11. The luminal microenvironments, which are comprised of a number of ions, organic solutes, and macromolecules, play important roles in the maturation and storage of spermatozoa in the epididymides, and threat the epididymal epithelium makes an optimal environment by absorbing electrolytes and water, as well as by secreting organic compounds12,13. The disturbed microenvironments might lead to a rise in the viscosity of the epididymal fluid and subsequent formation of plugs obstructing the epididymal duct14,15.

The present study showed that the accumulation of spermatozoa was seen in L-Cys-treated adult rats at study weeks 2, 3, and 4, in keeping with the incidence in L-Cys-treated pubertal rats at study week 4 (Table 2); this indicated that the administration of a large amount of L-Cys might have disturbed the luminal microenvironments not only in adults but also in pubertal rats. However, the occurrences of small ducts, dilated ducts and interstitial edema were rarely observed in L-Cys-treated adult rats, and sperm granuloma was seen only in one L-Cys-treated adult rat. Besides age-dependent decrease in incidence of L-Cys-induced rat sperm granulomas, these findings indicate that a rise of viscosity of the epididymal fluid might be not so severe that the duct could be ruptured in the adult rats. Therefore, the prolonged maturation of epididymal ducts, characterized by the presence of small ducts, might be responsible for the development of sperm granulomas in L-Cys-treated pubertal rats.

Vasectomized men occasionally develop sperm granulomas, suffering from persistent and troublesome pain, and discomfort, all of which may be associated with distention and leakage of the epididymides and vas deferens3. The local effects of vasectomy on the reproductive tract in humans are not known in detail, and the pathogenesis of sperm granulomas, particularly in terms of the process of leakage of sperm and the role of macrophages appearing, remains to be fully investigated3. The L-Cys-induced sperm granulomas in pubertal and adult rats may become a useful animal model for the pathogenesis on the human sperm granuloma.

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References


