PREVENTION OF DEVELOPMENT OF POST-TRAUMATIC ATROPHY OF THE RAT TESTIS BY INJECTION OF ASPIRIN

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The left testis was punctured with a needle (diameter 3 mm) in 42 sexually mature Wistar rats. The development of atrophy of the testis was observed 12 days after the operation in control animals receiving "empty" suppositories: the weight of the injured organ was greatly reduced, the seminiferous tubules of the whole testis were empty, their spermatogenic epithelium had undergone degeneration or destruction, the permeability of the blood-testicular barrier (BTB) for endogenous globulins was increased, and sharp changes were found in the ultrafine structure of the principle components of the barrier – the tunica propria of the seminiferous tubules and the Sertoli cells. After injection of aspirin (0.5 g daily for 5 to 12 days) into the rats the development of post-traumatic atrophy of the testes was not observed. The aspirin had no effect on the character and intensity of pathological processes developing in the focus of injury, but it prevented the spread of destructive changes to the intact part of the testis and disturbance of the permeability of the BTB away from the region of injury. The effect of aspirin on the development of post-traumatic testicular atrophy is evidently connected with its inhibitory action on prostaglandin synthesis.

KEY WORDS: aspirin; testicular atrophy; blood-testicular barrier.

Trauma of the testis in mammals gives rise to the development of permanent aspermatogenesis as a result of a rapidly developing inflammatory reaction, affecting the whole organ [3-5, 8, 9]. Characteristic features of the commencing inflammatory process in the testis after trauma are increased permeability of the blood-testicular barrier (BTB) relative to endogenous globulins, migration of cells of the plasma cell and lymphoid series into the interstitial tissue, and subsequent destruction of the spermatogenic epithelium [2, 5]. There is evidence [11] to suggest that during the development of its inflammatory reaction the prostaglandins serve as mediators. Aspirin has been shown to inhibit prostaglandin synthesis in laboratory animals and man [7].

The object of this investigation was to study the effect of aspirin on the development of post-traumatic testicular atrophy.

EXPERIMENTAL METHOD

Experiments were carried out on 42 sexually mature male Wistar rats weighing 330-400 g. The left testis of all the animals was punctured with a needle 3 mm in diameter in a direction perpendicular to the long axis of the organ. The a. spermatica propria remained intact. The operations were performed under sterile conditions and under light ether anesthesia. The experimental rats received aspirin per rectum in a dose of 0.5 g 1 h before the operation. Later the rats received the same dose of aspirin once a day for

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TABLE 1. Effect of Aspirin on Changes in Weight of the Testis 12 Days after Puncture of the Organ (M ± m)

Character of treatment	Testis	Weight of testis (in % of body wt.)	P
Puncture of testis + "empty" suppositories (control)	Intact Injured	4,67±0,12 3,06±0,23	<0,001
Puncture of testis + aspirin (5 days)	Intact Injured	4,43±0,15 4,21±0,25	0,29
Puncture of testis + aspirin (12 days)	Intact Injured	5,01±0,22 4,49±0,27	0,134

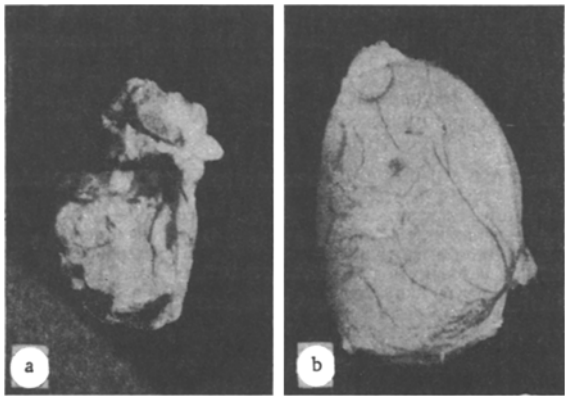

Fig. 1. General appearance of the rat testis 12 days after puncture of the organ: a) control; b) injection of aspirin (12 days).

4 days (group 1, 8 animals) or for 11 days (group 2, 13 animals). A corresponding number of suppositories not containing aspirin ("empty") was given to 21 control rats. All the rats were decapitated 12 days after the operation. The testes were fixed in Bouin's fluid and paraffin sections, 5μ in thickness, were stained with hematoxylin–eosin. Prefixation of the material for electron microscopy was carried out with glutaraldehyde, followed by fixation with buffered OsO_4 solution, dehydration, and embedding in Epon-Araldite mixture. Sections were cut on the LKB ultratome, stained with uranyl acetate and zinc citrate [10], and examined in the Tesla BS-516 electron microscope. The permeability of the BTB to endogenous globulin was determined in 4 rats from each group chosen at random [1]. The statistical analysis of the results was carried out by the Fisher–Student method.

EXPERIMENTAL RESULTS

The control rat developed post-traumatic atrophy of the testis. During the experiment the weight of the injured testes decreased considerably compared with the weight of the intact testes of the same animals (Table 1). In 11 of the 21 rats the weight of the injured testis fell by more than 50%. The injured testes were livid in color, flabby in consistency, and irregular in shape because of adhesions with the surrounding tissues (Fig. 1a). Under the light microscope, the seminiferous tubules of the whole organ were empty in testes whose weight was sharply reduced after the operation, the walls of the tubules were destroyed, the cytoplasm of the Sertoli cells was vacuolated, seminal granulomas formed, and the interstitial tissue was infiltrated by many lymphocytes. Electron microscopy revealed folding of the acellular layers of the tunica propria of the seminiferous tubules, and changes in shape of the cell nuclei of the inner and outer layers of the tubular membrane (Fig. 2e). Many vacuoles and fragments of degenerating spermatids, with partly destroyed tails, were seen in the cytoplasm of the Sertoli cells. Deep invaginations were present in the nuclei of the Sertoli cells and spermatogenic epithelium and the chromatin was distributed mainly peripherally.

Investigation of the permeability of the BTB revealed fixation of the globulins by individual cells of the spermatogenic epithelium and luminescence of conglomerations of degenerating cells, and also of interstitial tissue throughout the sections through the injured testes. In the intact testes no penetration of globulins within the seminiferous tubules was observed. Pathological changes were absent in the microscopic structure of the intact testes.

After administration of aspirin for 5 or 12 days to the animals no significant decrease in weight of the injured testis was observed compared with the intact testis (Table 1). The difference between the weight of the injured testes in the rats of groups 1 and 2 was not significant ($P < 0.001$). The injured testes were indistinguishable in shape, color, and consistency from the intact organs. They were not adherent to the surrounding tissues and the site of puncture was easily distinguished by the presence of a few whitish tubules (Fig. 1b). Under the light microscope infiltration of the interstitial tissue and walls of the tubules

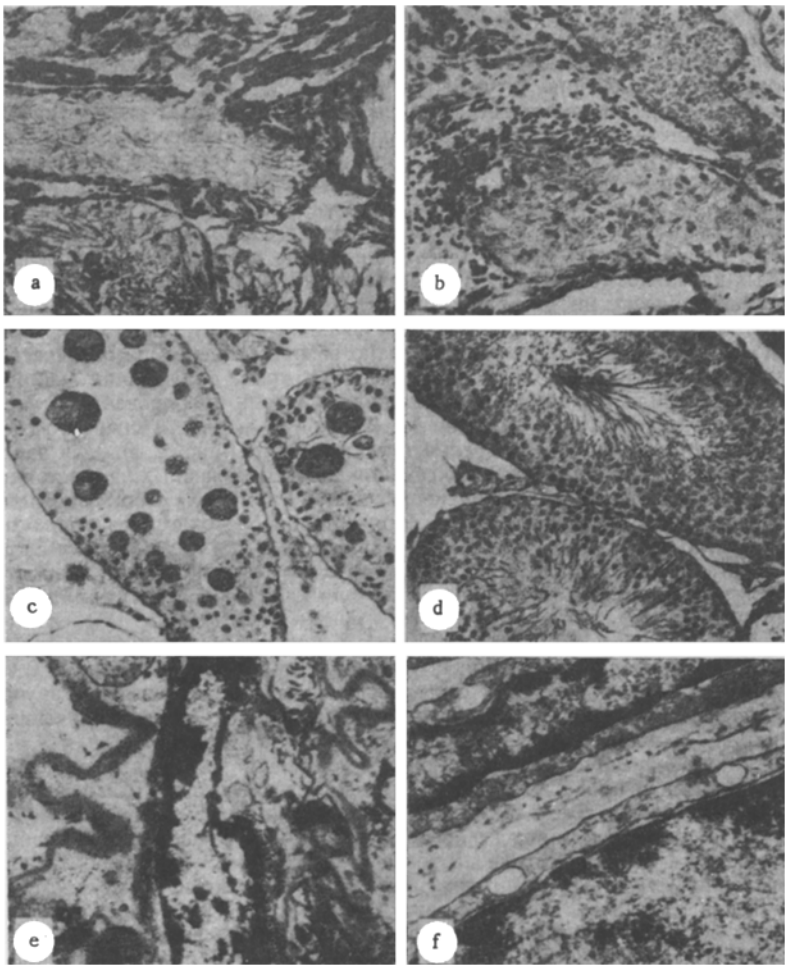

Fig. 2. Rat testis 12 days after operation: a) diffuse aspermatogenesis, destruction of wall of seminiferous tubules away from the region of injury in testis of a control rat; b) cytolysis of sex cells, infiltration of tubular wall and interstitial tissue by lymphocytes in focus of injury of the testis in a rat receiving aspirin for 12 days; c) multinuclear spermatids in region of injury in testis of the same rat; d) active spermatogenesis and absence of pathological changes in interstitial tissue outside the focus of injury in testis of the same rat; e and f) ultrastructure of tunica propria of seminiferous tubules located some distance from site of puncture; e) control: folding of acellular layers, disorganization of collagen fibers, change in shape of nucleus, and redistribution of chromatin in myoid cell; f) experiment: normal structure of tunica propria of a tubule, a,b,c,d) hematoxylin–eosin, 200×; e) 4800×; f) 13,370×.

by lymphocytes (Fig. 2b) and the formation of multinuclear spermatids (Fig. 2c) and seminal granulomas could be seen in the region of puncture. However, the pathological changes in the rats receiving aspirin did not spread outside the focus of injury. Remote from the site of puncture the tissue of the injured testis had the normal structure: spermatogenesis was active in the tubules and mature Leydig's cells were present in the interstitial tissue (Fig. 2d). Electron-microscopic studies showed an unchanged structure of the tunica propria of the seminiferous tubules in areas of the testis some distance from the site of puncture; all 4 layers of the tunica propria lay strictly parallel to each other and had all the characteristic features of their complex organization (Fig. 2f). No disturbances were found in the ultrastructure of the spermatogonia, spermatocytes, spermatids, and Sertoli cells.

The permeability of the BTB for endogenous globulins was indistinguishable from that in intact testes in areas of the injured testis away from the site of injury. No pathological changes were found in the intact testes of the rats receiving aspirin.

Administration of aspirin to rats, both for a short (5 days) and a long period (12 days) thus prevented the development of post-traumatic testicular atrophy. The results indicate that aspirin does not prevent the onset of the fundamental components of the testicular response to trauma characteristic of an autoimmune lesion of the testis, but it does prevent these changes from spreading to the uninjured part of the gland. In previous investigations [2, 4-6] the writers showed that a leading factor in the pathogenesis of autoimmune destruction of the testis caused by trauma or immunization with its antigens mixed with Freund's complete adjuvant is increased permeability of the BTB of the whole organ. The results described above indicate that following administration of aspirin changes in the permeability of the wall of the seminiferous tubules remote from the site of injury with respect to endogenous globulins do not take place in the injured testis, whereas in control rats the same conditions caused globulins to penetrate into all the tubules of the testis. Presumably it is at this stage of development of the pathological process in the injured testis that aspirin exerts its inhibitory action. The results now obtained, showing that aspirin prevents increased permeability of the BTB in response to testicular injury, are in agreement with recently published results [11] showing that aspirin inhibits the increase in protein concentration in the fluid of the anterior chamber of the rabbit eye observed after injury to the eye. Considering that injection of prostaglandins leads to the development of an inflammatory process in the eye, the workers cited postulated that eye injury stimulates the synthesis of prostaglandins, which cause vasodilatation, alter the permeability of barrier structures, and cause the subsequent development of inflammatory changes in the injured eye. A similar mechanism must evidently lie at the basis of the pathogenesis of post-traumatic atrophy of the testis and the prevention of its development by aspirin is linked with the inhibition of prostaglandin synthesis.

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