Inhibitory Effects on Cadmium-Induced Testicular Damage by Pretreatment with Smaller Cadmium Dose

By

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In recent years it has repeatedly demonstrated by a number of investigators in various animal species that a single administration of cadmium salt produces a selective damage to the testis (in mice, Meek 1959, Chiquoine 1964; in rats, Pařízek and Záhoř 1956, Allison and Deanesly 1962, Kar and Das 1962, Gunn, Gould and Anderson 1961, 1963a, b, Mason, Brown, Young and Nesbit 1964; in rabbits, Cameron and Foster 1963). It has also been known that the testis injury caused by cadmium can be prevented by zinc salts simultaneously administered (Pařízek 1956, 1957; Gunn, Gould and Anderson 1961, 1963a). In the course of studying morphological alterations of the testis and accessory sex organs following cadmium administration in young mice, it was found that the damage-inducing effect of cadmium on the testis was inhibited when the mice had been treated with a smaller dose of cadmium prior to the administration of the effective dose of cadmium. So far as we know, such an inhibitory effect on cadmium injury to the testis by pretreatment with a smaller dose of the same substance has not been reported.

Material and Methods

The present study was performed using male mice of Japanese dd-strain which is most commonly used in Japan. The animals were fed on a diet of commercial mouse pellets (NMF, Oriental Co. Japan) and water ad libitum, and maintained at constant environmental conditions. The mice were divided into six groups, one control and five experimental groups, each of which consisted of 40 mice (Table 1). Control group was composed of normal, untreated males. For
Group 1, a single subcutaneous injection of 0.1 ml of 0.1% CdCl₂ in distilled water for each animal was given at 4 weeks of age. In Groups 2, 3, 4 and 5, animals were treated respectively with the following progressively reduced doses of cadmium chloride at 26 days of age, and then two days later, that is, at 4 weeks of age they further received the same dosage of cadmium chloride as administered for Group 1. For the pretreatment of Groups 2, 3, 4 and 5, 0.1 ml of 2-, 4-, 8- and 16-fold diluted solution of 0.1% CdCl₂ was subcutaneously injected for each mouse, respectively. Thus, Groups 2, 3, 4 and 5 were pretreated with a half, one-fourth, one-eighth and one-sixteenth of the dosage administered at 4 weeks of age, respectively. All the animals from control and experimental groups were killed at 9 weeks of age. At autopsy, after the body weight was recorded, the testes and seminal vesicle were removed as promptly as possible, and then weighed on a torsion balance to the nearest mg. When there was any difference in testis weight between both sides, which, if any, was very slight, the weight value of the heavier side was taken as the testis weight. The testes were then immediately fixed in fixatives, such as Bouin or Zenker fluids. The tissues were embedded in paraffin and serially or semi-serially sectioned at 5 μ. The sections were stained with hematoxylin and eosin and sometimes with PAS plus hematoxylin or with Heidenhain's azocarmine-aniline blue.

Results

1. Testis weight

The testis weights of all mice, both control and experimental, were plotted for each group in Fig. 1. The testes of all the normal untreated control were well developed, weighing, on an average, 95.6 ± 13.9 mg. The testes of experimental mice, on the other hand, varied markedly in weight from group to group. It was previously observed that in male mice receiving a single injection of a 0.1 ml of a 0.1% solution of CdCl₂ at 4 weeks of age a rapid selective destruction of the testis was induced due to a massive hemorrhage in more than 80% of the cases (Sawauchi 1965). At the level of one and a half dose mice often died immediately. Thus a dosage of 0.1 ml of a 0.1% solution of CdCl₂ was taken as a dose effective in inducing the testicular damage. In Group 1 consisting of mice receiving a single injection of the dose of CdCl₂ at 4 weeks of age, the testes were generally extremely atrophied and markedly small in weight as compared with those of normal controls. In Groups 2
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Fig. 1. Testis weights in control and experimental groups. C, control group; 1-5, experimental groups.

and 3 receiving the same dose of cadmium after pretreatment with one-half or one-fourth of the dose, respectively, atrophied testes were far fewer in number than in Group 1, and many of the testes weighed at or near the normal level. In Groups 4 and 5 given 0.1 ml of 0.1% solution of CdCl₂ following pretreatment with one-eighth or one-sixteenth of the dose, respectively, atrophied testes were again increased in number. Particularly in Group 5, the distribution of the testis weights was almost the same as that in Group 1. On a weight basis the testes in the experimental groups were divided into three classes: testes weighing more than 60 mg were classified as normal; those between 60 and 25 mg, subnormal; those less than 25 mg, atrophic, respectively. Table 1 presents the numbers of testis of each weight class in experimental and control groups. As seen in this table, atrophic testes occupied 82.5% (33/40) in Group 1, whereas they were 20% (8/40) and 25% (10/40) in Groups 2 and 3, respectively. In the latter two groups the normal and subnormal testes were increased in number. Difference in number of atrophic testis between Groups 1 and 2 or 3 was statistically significant (P<0.01). In Group 4 the percentage of atrophic testis was increased up to 52.5% (21/40) and in Group 5 it was the same as in Group 1. From these results it may be said on a weight basis
of the testis that cadmium-induced testicular damage is reduced or inhibited when mice have been pretreated with a half or one-fourth of the administered dose, but that pretreatment with one-eighth of the dose is less effective and pretreatment with one-sixteenth, no longer effective, in inhibiting the induction of the testicular damage.

2. Seminal vesicle weight

The seminal vesicle weight is generally used as an assessment of testicular androgen activity. The vesicle weights for each of the control and experimental groups are presented in Fig. 2. In comparison with Fig. 1, it may be seen that in each group the distribution of the seminal vesicle weights is, on the whole, similar to that of the testis weights. The seminal vesicles were also classified, on a weight basis, into three classes; normal (more than 70 mg), subnormal (70–30 mg) and atrophic (less than 30 mg). The number and percentage of each class for each group are presented in Table 1. In each of the experimental groups the frequency of atrophic vesicle was lower than that of atrophic testis. This was, as described later, due to quantitative difference of Leydig interstitial cells in atrophic

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* Group 1-5: experimental groups.
** 0: no treatment; I: a single injection of 0.1 ml of 0.1% CdCl₂ at 4 weeks; 1/2, 1/4, 1/8, 1/16: at the same dosage after pretreatment with 1/2, 1/4, 1/8, or 1/16 of the dosage, respectively.
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3. Histological findings of the testis

As mentioned above, the testes of the experimental mice were divided, on a weight basis, into three classes; normal, subnormal and atrophic. The testes from the normal weight class showed almost no histological changes as compared with those of the normal controls. In sections, the seminiferous tubules were closely packed, testes. The atrophic testes generally showed severe destruction of the seminiferous tubules, and were replaced by fibrous connective tissue. Leydig cells, however, varied considerably in different cases. Some cases of the atrophic testes were almost lacking in Leydig cells, but some others contained the cells in varying amounts. The vesicles in the former cases were atrophic, while those in the latter were subnormal or normal in weight in proportion to the amount of Leydig cells. Testes within normal or subnormal ranges of weight contained large numbers of Leydig cells like those of the control. Therefore, the seminal vesicles in cases with such testes were always at normal level in weight. From the level of testicular hormone activity, as assumed from the seminal vesicle weight, it may also be suggested that cadmium-induced testicular damage is inhibited by pretreatment with appropriate dose of the same substance.
and showed normal spermatogenesis. The interstitial tissue contained large amounts of Leydig cells which were usually seen in small clumps in angular spaces between the tubules (Fig. 3). The testes from the subnormal weight class were within a relatively wide range in weight, and showed varying degrees of histological changes. In this weight class, the testes with relatively large weight had relatively abundant seminiferous tubules, but the spermatogenesis was often inhibited in a greater or lesser degree. Some of the tubules were atrophic, and their spermatogenic epithelium was arranged in two to five layers without any mature sperm cells. The smaller testes of subnormal weight class showed severer tubular damage (Figs. 4 and 5). Most of the tubules were dispersed and disorganized without any mature sperm, and their wall sometimes consisted only of Sertoli cells. In the testes from the subnormal weight class, the interstitium was relatively wide between the tubules, and contained large amounts of Leydig cells (Fig. 5). The cells usually were arranged in large masses between the dispersed tubules. They were the same in structure as those of the normal testis. The large, polygonal cells possessed abundant eosinophilic, more or less vacuolated cytoplasm and large, spherical or often wrinkled nuclei. As mentioned above, in mice with testes of subnormal weight class, their seminal vesicles were always of normal weight. This would be explained by abundance in Leydig cells. The atrophied testes weighing less than 25 mg histologically showed severe damage. They were, for the most part, replaced by fibrous tissue (Fig. 6). In some cases, many of the seminiferous tubules disappeared and some remained only as necrotic remnants. In some testes, small amounts of tubules remained, but their epithelium consisted only of one to three rows of spermatogonia, spermatocytes and Sertoli cells. In the atrophied testes, Leydig cells varied in amount from case to case. In some cases there occurred only small amounts of the cells beneath the testicular capsule, and still in others there were large amounts of the cells (Figs. 7 and 8). When in small amounts, Leydig cells were relatively small in size and their cytoplasm was less eosinophilic. When present in large amounts, the cells were usually grouped in huge masses or clusters and their cytological details were the same as those of the normal testis. In cases with atrophic testes, the seminal vesicles were not always atrophied, as already shown. The vesicle weights were dependent of the amount of Leydig cells. In cases with almost no Leydig cells, the vesicles were also atrophied. In cases with small amounts of Leydig cells, the seminal vesicle
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weights were usually at the subnormal level. In cases where the testis, though atrophic, contained large amounts of Leydig cells, the seminal vesicle weight was at the level of the normal control. Consequently, even in the same group the number of cases with atrophic testis was larger than that of cases with atrophic testis was larger than that of cases with atrophic vesicle (Table 1). In addition, the atrophic testes from the pretreated groups, on the whole, contained Leydig cells in more abundance and more frequently than those from the group receiving a single dosage of cadmium at 4 weeks.

Discussion

From the previous and present results it is clear that cadmium produces a selective damage to the testis. At present, however, the mechanism of the specific action of cadmium on the testis is not yet quite clear. There have been two concepts as to whether cadmium affects primarily the testicular tubules or vessels. Considering that zinc is known to be essential to the complete development and maturation of sperm in the tubular epithelium and that the administration of large doses of zinc together with cadmium protects the testes against cadmium injury, it was previously inferred that cadmium replaces zinc in the spermatogenic epithelium and causes a primary damage to the tubular elements (Parízek 1956, 1957). This concept is attractive, but cannot adequately explain all the findings relating to cadmium testicular damage, as has recently been pointed out by Mason, Brown, Young and Nesbit (1964). It is more recently advanced that the primary effect of cadmium on the testis is vascular. Gunn, Gould and Anderson (1963a) noted in the rat that not only the testis but also the proximal end of the caput epididymis is specifically damaged by cadmium in virtue of injury to their vascular supply. In a light and electron microscopic study of the early changes which occurred in cadmium necrosis of the testis of the mouse, Chiquoine (1964) stated that, since the earliest alterations prior to cell damage in the tubular epithelium were observed in the capillary endothelium, the primary site of action of cadmium was the endothelium of the vascular bed in the testis. Subsequently Mason, Brown, Young and Nesbit (1964) also considered the unusual sensitivity of the testis to cadmium to be related to unique features of its vasculature. Most recently Maekawa, Tsunenari, Nokubi and Waki (1965) stated in the rat that the earliest changes of the testes following cadmium
administration were found in the capillary endothelium and that the testicular damage caused by cadmium was due to a primary disturbance of the testicular vasculature. In addition, these investigators suggested on a basis of the weight of androgen-sensitive organs that a temporarily enhanced discharge of androgen from the testes occurred shortly after cadmium administration. We also observed, in agreement with the previous investigators, that cadmium necrosis of the testis of the mouse was caused as a result of massive hemorrhage which occurred following cadmium administration (Sawauchi 1965). As shown in the present results, the specific effects of cadmium on the testis could be inhibited or reduced when the animals had been pretreated with a half or one-fourth of the dose of cadmium. Cadmium dosages as small as a half or one-fourth of the dose alone were insufficient to produce massive hemorrhage and consequent necrosis of the testis, but could prevent the testis from being injured by cadmium subsequently administered. If, as has recently been suggested, the specific sensitivity of the testicular vessels to cadmium is assumed to be responsible for inducing massive hemorrhage, it will of necessity be affected and reduced by the pretreatment with smaller doses of cadmium. As the dose used for pretreatment was further reduced, the inhibitory effect became less prominent. For example, the pretreatment with one-sixteenth of the necrosis-inducing dose was no longer effective in inhibiting the testicular damage. Such small dosages of cadmium might be insufficient to affect the specific sensitivity of the testicular vessels to cadmium. Thus adequate dosages of cadmium seem necessary to protect the testis against the subsequent cadmium.

It is also of interest that in some atrophic testes, particularly in Groups 2 and 3, although the seminiferous tubules were very severely damaged, there were found large amounts of Leydig cells. This indicates that Leydig cells alone could remain or regenerate even after severe tubular damage. Thus it is possible that, if cadmium is successively given at certain dosages, Leydig cells alone remain separately from the seminiferous tubules. This problem is now being investigated in our laboratory in relation to experimental production of hyperplastic and neoplastic proliferation of Leydig cells.
Summary

In male mice receiving a single injection of cadmium chloride at a dosage of 0.1 ml of a 0.1% solution in distilled water at 4 weeks of age, the testes were selectively damaged. At 8 weeks of age the testes were found extremely atrophied in 82.5% of the cases examined (33/40), being very small (less than 25 mg) in weight. On the other hand, when mice were given cadmium in the same way two days after pretreatment with as small as a half or one-fourth of the dosage, the testicular damage was inhibited. At 8 weeks the atrophied testes were significantly fewer in pretreated mice than in those given a single injection of cadmium without any pretreatment. As cadmium dose given for pretreatment was further reduced, the inhibitory effect became decreased. The inhibitory effect by the pretreatment was also evident on the basis of seminal vesicle weight reflecting the level of testicular hormone activity. The testes histologically underwent varying degrees of structural changes in parallel to the weight changes. In the atrophied testes the seminiferous tubules were severely damaged and extremely reduced in amount, but Leydig cells varied in amount from case to case. The atrophied testes from the pretreated groups contained Leydig cells in abundance more frequently than those from the group receiving a single dosage of cadmium without any pretreatment.

References

Explanation of Figures

Fig. 3. Testis of a case from Group 2. In this case the testis weighed 111 mg and the seminal vesicle weight was 172 mg. The seminiferous tubules and interstitial tissue are normal in appearance. Bouin fix., hematoxylin-eosin. ×140

Fig. 4. Testis of a case from Group 2. The testis weighed 30 mg (subnormal) and the seminal vesicle weight was 178 mg (normal). The testis shows severe tubular damage, but the interstitial tissue contains large amounts of Leydig cells. Bouin fix., hematoxylin-eosin. ×56

Fig. 5. The same as in Fig. 4. ×140

Fig. 6. Testis of a case from Group 1. The testis weighed 12 mg (atrophic) and the seminal vesicle weighed 22 mg (atrophic). The parenchymal tissue is almost replaced by fibrous tissue with almost no Leydig cells. The remaining seminiferous tubules appear necrotic. Bouin fix., hematoxylin-eosin. ×56

Fig. 7. Testis of a case from Group 3. The testis weighed 20 mg (atrophic) and the seminal vesicle weighed 142 mg (normal). The seminiferous tubules are severely damaged. The interstitial tissue is relatively wide and contains large amounts of Leydig cells. Bouin fix., hematoxylin-eosin. ×56

Fig. 8. The same as in Fig. 7. Large amounts of Leydig cells are seen in the interstitial tissue. ×280
Plate I

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 7.

Fig. 8.

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