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Effects of carboplatin on the testis

A histological study

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Summary. In the present study, the influence of carboplatin [diammine(cyclobutane-1,1-dicarboxylato)platinum(II)], the main and most active representative of second-generation antitumour platinum complexes, on the morphology of the testes of male CF1 mice was investigated histologically by examining semithick sections. Carboplatin was administered in doses of 30, 60 or 120 mg/kg and applied as a single intraperitoneal injection. For comparison purposes, the parent compound cisplatin [*cis*-diamminedichloroplatinum(II)] was administered at equitoxic doses (3, 6 or 12 mg/kg). At various intervals between days 1 and 28 after treatment, the testes were removed and embedded in Epon. Both compounds effected severe structural alterations of Sertoli cells, disrupted the blood/testis barrier, and impaired the processes both of spermatogenesis and spermiohistogenesis. The structural damage in the testes following treatment with carboplatin was at least as pronounced as that occurring under the influence of equitoxic doses of cisplatin. Within a few days, the intercellular spaces around Sertoli cells widened, the tight contacts with neighbouring cells were disrupted, the cytoplasm of Sertoli cells disintegrated and their nuclei shrank. Numerous necroses, abnormal mitotic figures of spermatogenic cells and malformed spermatozoa appeared. Severe damage was evident on days 10–21 after treatment with carboplatin, the strength of the symptoms being clearly dependent on the dose applied. The first indications of ongoing recovery processes were detected on day 21 in the case of the low dose (30 mg/kg) or on day 28 following treatment with 60 mg/kg or 120 mg/kg. These results confirm that carboplatin is at least as toxic to the testes as cisplatin and that its substitution for cisplatin in clinical therapy does not diminish the problem of drug-induced infertility following platinum-based chemotherapy.

Introduction

Since the detection of antitumour properties of some platinum compounds by Rosenberg and coworkers in 1969 [20], the inorganic complex cisplatin [*cis*-diamminedichloroplatinum(II)] has been confirmed to be one of the most active cytostatic drugs available today for the clinical treatment of human solid carcinomas [21, 25]. Numerous studies have revealed that urogenital carcinomas are obviously most sensitive to the antitumour action of cisplatin [21, 25]. Testicular carcinomas especially of both the seminoma and non-seminoma type, are effectively treatable by cisplatin-based chemotherapy and curable in many cases even at the advanced, disseminated stage. However, as cisplatin is burdened by severe side-effects, such as nephrotoxicity, neurotoxicity, gastrointestinal irritation and a profound disturbance of the patients' general behaviour, the clinical administration of cisplatin can be actually limited by the interference of non-tolerable side-effects [26]. Thus, second-generation platinum complexes have been developed in order to enlarge the therapeutic spectrum and to diminish drug-induced side-effects of platinum-based chemotherapy [7, 19]. The main representative of these compounds has been revealed to be carboplatin [diammine(cyclobutane-1,1-dicarboxylato)platinum(II)], which exhibits a similar spectrum of activity to that of cisplatin, but is characterized by a quite different pattern of toxicity, lacking pronounced nephrotoxicity, neurotoxicity and emesis. Bone marrow suppression is generally found to be the dose-limiting adverse toxic effect of carboplatin [3, 19]. As it reduces the general behaviour of treated patients to a much less extent than cisplatin and is, thus, better accepted by the patients, carboplatin increasingly substitutes for cisplatin in clinical chemotherapy of diverse cancer diseases [1, 2, 4, 12, 15, 27].

It is known from previous studies that cisplatin severely damages the structure and function of the testes, deeply impairs the processes of spermatogenesis and reduces the secretion of testosterone by the interstitial cells of Leydig [6, 17, 18, 24]. Azoospermia or oligozoospermia and infertility are the consequences in human patients at least for

several years after therapy [6]. As most patients who fall sick with testicular carcinoma are younger than 35 or 40 years and have a high chance of being cured by platinum-based therapy even when the disease is disseminated, the injury of testicular structure and function by cisplatin must be considered as a severe side-effect, which deeply impairs the reproductive potency of the patients following successful therapy.

No information is yet available concerning the influence of carboplatin on the testis, though it increasingly replaces cisplatin in clinical chemotherapy. As it would be very favourable to have a cytostatic drug active against cancer disease, especially against testicular carcinomas, which does not damage the normal testicular tissue, we investigated in the present study the influence of carboplatin on the histological appearence of the testis of mice in comparison to cisplatin applied at equitoxic dose levels.

Materials and methods

Male CF1 mice, purchased from Winkelmann, Paderborn, FRG, were kept under standard conditions and received food (Altromin) and tap water ad libitum. At the beginning of the experiments, they were 12–14 weeks old, weighed about 27–30 g, and were treated with single intraperitoneal injections of diammine(cyclobutane-1,1-dicarboxylato)platinum(II) (carboplatin), purchased from Heraeus, Hanau, FRG. For comparison purposes, an equal number of animals received single-dose injections of the parent compound *cis*-diamminedichloroplatinum(II) (cisplatin), obtained from Strem Chemicals, Karlsruhe, FRG. The compounds were dissolved in a mixture of dimethylsulphoxide and saline (1/9, v/v) in such a manner that 0.02 ml/g body weight was applied. Both compounds were administered at three equitoxic dose levels of 30, 60, or 120 mg carboplatin/kg and 3, 6, or 12 mg cisplatin/kg. The toxic thresholds, previously determined in our laboratory with male mice of the same strain and identical age, amounted to 130 mg/kg or 13 mg/kg (LD_{10}) and 140 mg/kg or 15 mg/kg (LD_{20}) in the case of carboplatin and cisplatin respectively. The animals were weighed daily in order to estimate general toxic effects.

At intervals of 1, 2, 4, 6, 8, 10, 15, 21, and 28 days after treatment, but not at later dates, two animals of each group were anaesthetized with Nembutal and perfused with a fixative solution. For this purpose, the thorax of the animals was opened, the apex of the heart cut off, and a cannula inserted into the aorta. The animals were then perfused for 30 min with a solution containing 3% glutaraldehyde and 3% paraformaldehyde in cacodylate buffer (pH 7.2). Thereafter, the testes were removed, immersed for 10 min in the fixative solution, cut into sector-like pieces, immersed for another 24 h in fixative solution, postfixed with a 1% solution of osmium tetroxide in cacodylate buffer, dehydrated and embedded in Epon. Semithick sections of 0.5–1 µm were prepared, mounted and stained with toluidine blue.

Results

The body weight of none of the animals treated with the low dose of cisplatin (3 mg/kg) or carboplatin (30 mg/kg) decreased during the observation period of 28 days. This confirms the subtoxic character of these doses. Applying the medium doses, there was again no effect observable in the case of carboplatin (60 mg/kg), but reversible weight losses of 5% (3%–6%) occurred following application of 6 mg cisplatin/kg. Regarding the high dose levels of both compounds (12 or 120 mg/kg), all animals treated lost between 10% and 18% (mean value 15%) of their initial body weight within 3–4 days. This indicates a corresponding impairment of general behaviour by both cytostatic drugs at this sublethal, high dose level. The reduced body weights remained stable for a further 8–12 days and then again increased slowly, without reaching the initial value until day 28.

Influence of cisplatin upon testicular morphology

Even the low dose of cisplatin (3 mg/kg) was sufficient to induce profound structural alterations within the testicular tissue of mice. Whereas the germinal epithelium of untreated seminiferous tubules consisted of densely arranged spermatogenic cells in different developmental stages (Fig. 1 a, b), the intraepithelial intercellular spaces enlarged and the contact between the cells loosened within 2–4 days after substance application. This was seen in the broad luminal area occupied by spermatids as well as in the basal layer around spermatogonia and Sertoli cells (Fig. 1 c). The course of the basement membrane encircling the seminiferous tubules became irregularly wound and was no longer straight at this time. Degenerating and necrotic cells appeared in the middle and luminal areas of the epithelium (Fig. 1 c). Its breadth was reduced and the number of spermatids and developing spermatozoa fell during the following days. Arrested mitotic figures were detected between and above primary spermatocytes, and giant cells with three or four nuclei were found more luminally among normal spermatids (Fig. 1 c). These morphological features were seen until day 15 without any tendency of improvement. Thereafter there were signs of recovery, manifest by a gradual closure of enlarged intercellular spaces in the basal layer of the epithelium, by a growing number of normal mitoses of spermatogonia and a gradually increasing width of the germinative epithelium. In the cytoplasm of Sertoli cells, numerous inclusion bodies, probably secondary lysosomes, appeared and, on day 21, a great number of abnormal mitotic figures with condensed chromosomes in a bizarrely coiled arrangement occurred luminally in the primary spermatocytes, which are easily identifiable by their typical prophasic nucleus (Fig. 1 d). Single necroses were still detectable in the luminal areas of the epithelium until day 28. These necroses presumably disappeared during the following days, but there are no exact data from the present study since no observations were made later than day 28 after drug application.

Following administration of the higher doses of cisplatin (6 and 12 mg/kg), there were alterations similar to those observed after the 3-mg/kg dose, the strength and velocity of appearance of the symptoms, however, being clearly dependent upon the dose applied. Within 1 day after treatment with 6 mg/kg or 12 mg/kg, the basally located cells became detached from the underlying basal lamina (Fig. 2 a), giving rise to the occurrence of an irregularly widened cleft between the basement membrane and the Sertoli cells and spermatogonia, which normally rest closely on it. Simultaneously, the first necroses appeared in the areas occupied by spermatids and maturing spermatozoa. They included abnormally condensed nuclei and/or vacuolized cytoplasms (Fig. 2 a, b). Thereafter, the inter-

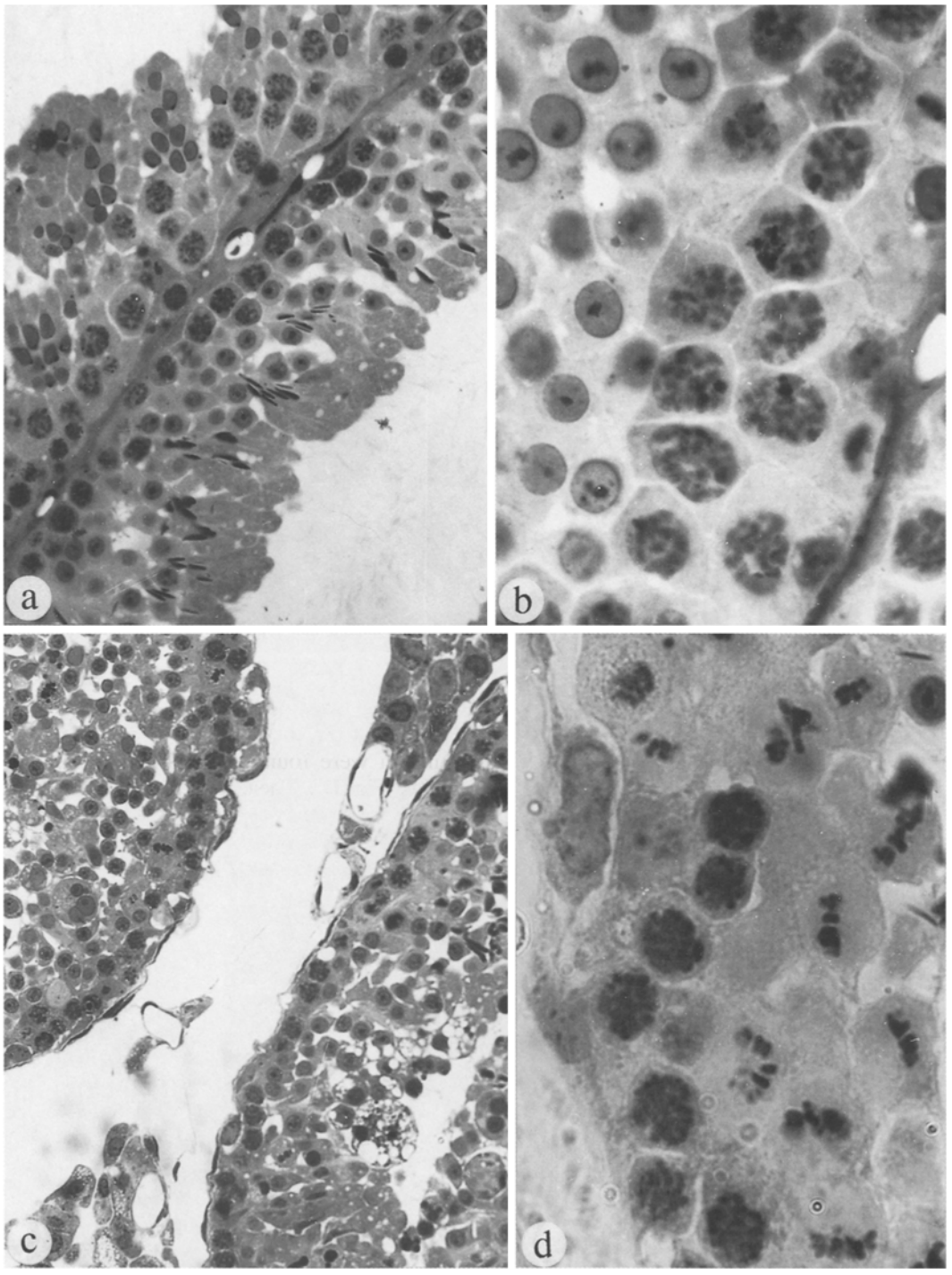

Fig. 1. Seminiferous tubules of untreated control animals (**a, b**) contain densely arranged spermatogenic cells and supporting Sertoli cells resting on the basal lamina. Following treatment with 3 mg cisplatin/kg, the intercellular spaces widen until day 4 (**c**) and numerous cells with condensed nucleus and vacuolized cytoplasm occur. On day 21 (**d**), many cells with abnormal mitotic figures are visible in the luminal region of primary spermatocytes. ×450 (**a**); ×1100 (**b**); ×410 (**c**); ×1200 (**d**)

cellular spaces became more and more enlarged throughout the whole germinative epithelium. On days 10–21 (Fig. 2c,d), many Sertoli cells were structurally damaged and often contained nuclei with clumped chromatin and shrinking cytoplasms. They retreated from neighbouring cells, their outer shape changing to star-like figures (Fig. 2c,d). The course of the basal lamina around the seminiferous tubules was remarkably irregular. Beginning on day 2 (12 mg/kg) or day 4 (6 mg/kg), numerous mitotic or, more probably, meiotic figures were obviously arrested in the metaphase and contained clumped chromosomes in coiled or irregularly dispersed arrangement. These cells occurred first in the region of primary spermatocytes and had slowly moved to the middle of the germinative epithelium by days 21 and 28 (Fig. 2d,e). Until then, the number of normally appearing primary spermato-

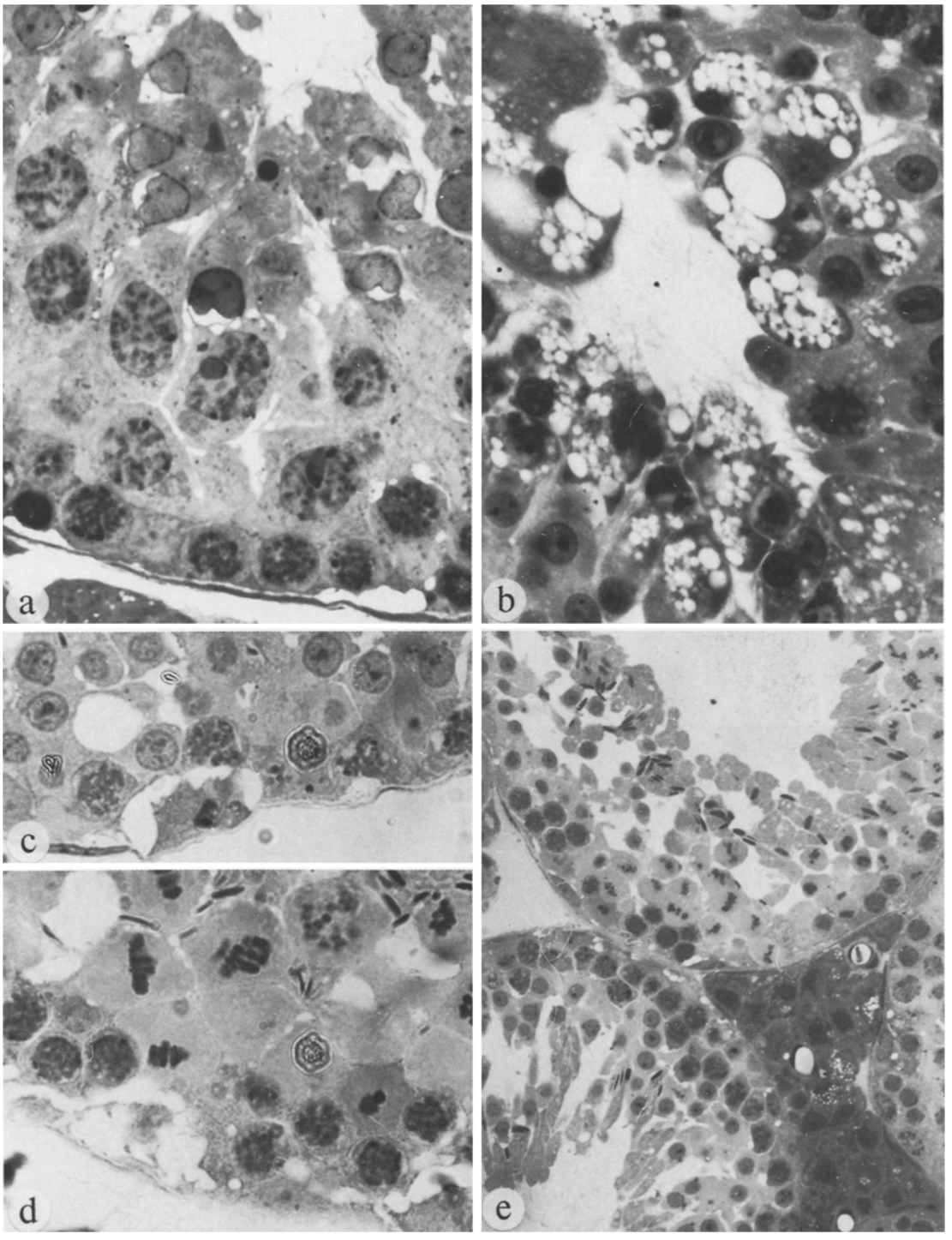

Fig. 2. Seminiferous tubules following application of 6 mg cisplatin/kg (**a, b, d, e**) or 12 (**c**) mg/kg. On day 1 (**a**), the cleft below the basal cell row and the intercellular spaces between spermatids begin to widen. The first necroses are detectable and become more frequent especially in the luminal region of the germinative epithelium during the following day (**b**). Until days 10 (**c**) to 21 (**d**), the intercellular spaces markedly enlarge in the basal areas also. On days 21 (**d**) and 28 (**e**), numerous abnormal mitotic figures of spermatogenic cells are remarkable. ×1100 (**a, b, c**); ×950 (**d**); ×450 (**e**)

cytes increased as an obvious hint of the onset of slow regeneration processes. Nevertheless, the germinative epithelium was still structurally altered at this time and contained fewer mature spermatozoa than normal, there being necrotic cells and abnormal giant cells in the luminal region. This still contained widened intercellular spaces, which mainly occurred between spermatids and around Sertoli cells, the structure of which had clearly normalized by day 28 (Fig. 2e).

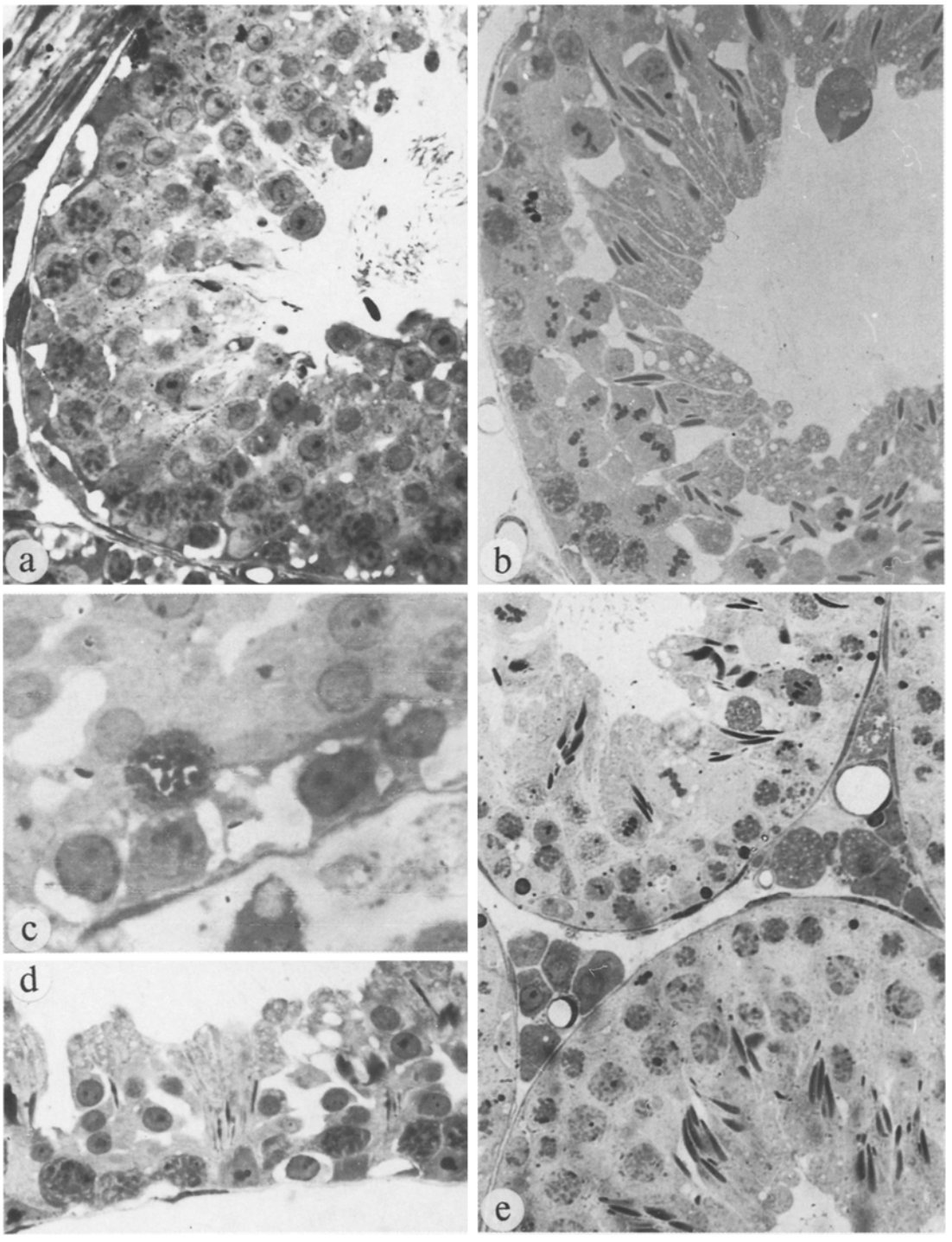

Fig. 3 a–e. Seminiferous tubules after treatment with carboplatin (30 mg/kg). On day 2 (**a**) widened intercellular clefts appear around the basally located Sertoli cells. The first necroses of spermatogenic cells are observable. Until day 6 (**b**), the intercellular spaces between spermatids markedly enlarge and numerous arrested mitotic figures appear in the spermatid zone. On days 10 (**c**) and 15 (**d**), many Sertoli cells contain a nucleus with condensed chromatin and a split and disintegrated cytoplasm. By day 21 (**e**), most intercellular clefts have closed again, but some arrested mitoses, necroses and abnormal spermatozoa are still present. ×500 (**a, e**); ×950 (**b, c, d**)

Influence of carboplatin upon testicular morphology

When carboplatin was applied at the low dose level (30 mg/kg), the intercellular spaces between and around the basally located cells enlarged within 2 days (Fig. 3a) in a more pronounced manner than in the case of cisplatin administered at an equitoxic dose (cf. Fig. 1c). Following treatment with carboplatin, the cytoplasm of many Sertoli cells became torn up and remained in this state until day 15 (Fig. 3b–d). During this period, the chromatin of some Sertoli cell nuclei shrank (Fig. 3c) and the course of the basement membrane located beneath them was remarkably winding (Fig. 3b–d). After day 2, malformed spermatozoa occurred in luminal regions and necrotic spermatogenic

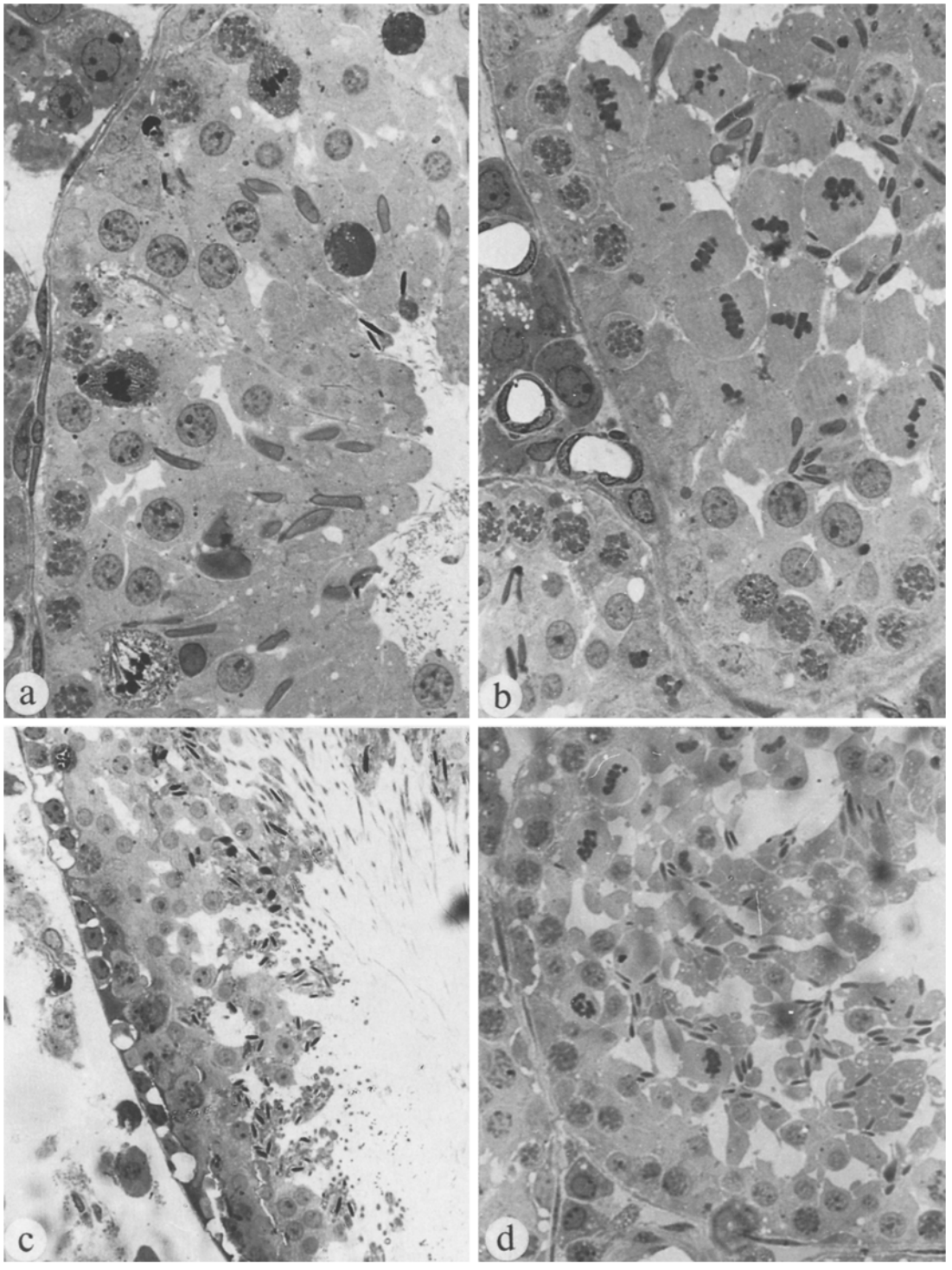

Fig. 4 a–d. Seminiferous tubules following administration of 60 mg carboplatin/kg. On day 1 (**a, b**) the first necroses and arrested mitotic figures occur in the layers of spermatocytes and maturing spermatids. Note the degenerating mitoses in **a** and the coiled mitotic figures in **b**. During the following days, the Sertoli cells become deeply injured and surrounded by widened intercellular spaces (**c**, day 15). Their cytoplasmic integrity is destroyed (**c**) and, simultaneously, the processes of spermiohistogenesis are obviously impaired leading to the appearence of malformed spermatozoa. On day 28, the seminiferous tubules are still structurally damaged, the intercellular spaces are still enlarged and some arrested metaphases are distributed between normal spermatids. ×950 (**a, b**); ×380 (**c**); ×480 (**d**)

cells were seen in the areas normally occupied by primary spermatocytes and spermatids (Fig. 3a–d). Simultaneously, the number of primary spermatocytes decreased and enlarged extracellular spaces perforated the epithelium, which had clearly become thinned within a few days (Fig. 3c). Beginning on day 21, the first signs of recovery were detectable and manifest by a gradual closure of the formerly enlarged intercellular spaces and a slowly increasing appearance of primary spermatocytes (Fig. 3d). Nevertheless, necrotic cells, malformed maturing sper-

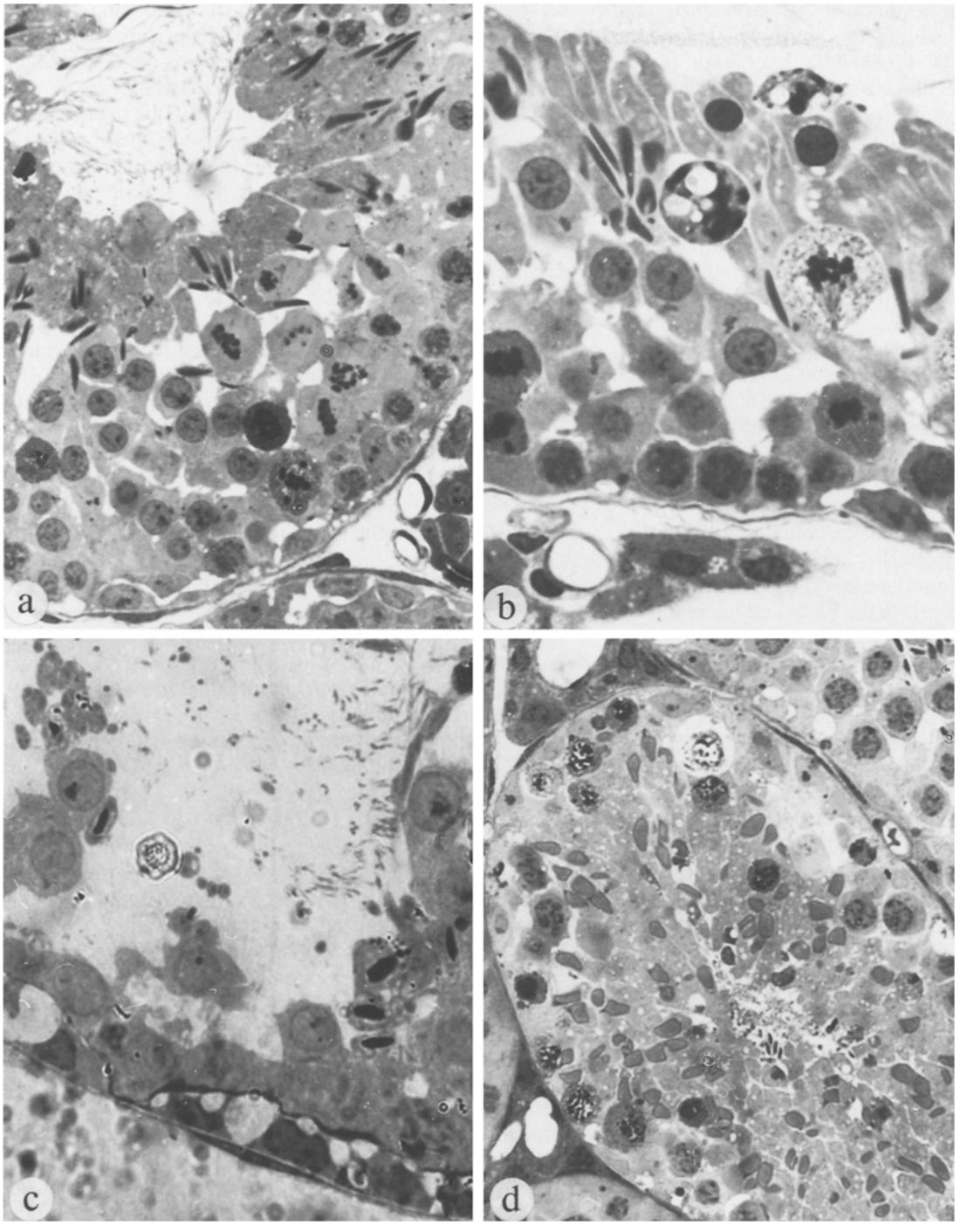

Fig. 5. Seminiferous tubules after treatment with 130 mg carboplatin/kg. As early as day 1 (**a, b**) the intercellular spaces are markedly widened throughout the whole germinative epithelium. Numerous arrested and, in some cases, degenerating mitoses are observable in the basal and middle layers. Simultaneously, the first necrotic cells occur. Until day 15 (**c**), the breadth of the epithelium becomes markedly reduced because of the loss of many spermatogenic cells. The structure of most Sertoli cells is severely damaged. On day 28 (**d**), numerous degenerating primary spermatocytes are still present and the local arrangement of spermatogenic cells in different developmental stages is still deeply disturbed. ×720 (**a**); ×950 (**b, c**); ×450 (**d**)

matids and abnormal mitotic figures, which were arrested in the mitotic or meiotic metaphase, remained visible until day 21 and later. Some Sertoli cells were still necrotic at this time, many of them including large secondary lysosomes (Fig. 3e). Since no animals were investigated later than day 28 after treatment, no information is yet available on whether these injuries were reversible and would disappear during the following days.

After the dose of carboplatin was increased to 60 mg/kg, the first histological changes had already occurred on day 1 and were manifest by a localized widening of the intercellular spaces in the middle and basal areas,

and by the occasional appearance of cellular necroses and degenerating mitoses within the germinal epithelium (Fig. 4a). Simultaneously, bizarrely coiled mitotic figures arose in the area of secondary spermatocytes and spermatids (Fig. 4b). During the following time until day 21, the intercellular spaces around the Sertoli cells enlarged and lacerations within the cytoplasm of the supporting cells occurred and increased markedly (Fig. 4c). The course of the basal lamina became winding and irregular. Simultaneously, the number of primary spermatocytes diminished, the width of the germinative epithelium irregularly decreased and cohering groups of spermatids and maturing spermatozoa containing bizarrely shaped nuclei sloughed off into the tubular lumen (Fig. 4c). On day 28, the first processes of recovery became observable though restitution was not yet achieved (Fig. 4d). The intercellular spaces began to narrow in the basal layers of the germinative epithelium, some normal mitoses of spermatogonia resting on the basal lamina were detectable, and spermiohistogenesis obviously normalized gradually.

By applying the high dose of carboplatin (130 mg/kg), similar, but more pronounced phenomena developed in comparison to the medium dose level. By day 1, the intercellular clefts were widened in the basal and luminal areas of the germinative epithelium (Fig. 5a,b). The supporting Sertoli cells began to retract from neighbouring cells, the course of the basal lamina became winding and numerous arrested mitoses as well as degenerating mitotic and necrotic cells were detectable in the areas of primary spermatocytes and spermatids (Fig. 5a,b). The processes of spermiohistogenesis were obviously disturbed, leading to the appearance of spermatids with pathologically condensed, round nuclei and spermatozoa containing abnormal nuclear figures and vacuolized cytoplasms. The occurrence of these phenomena increased during the following days and resulted in the appearence of pronounced structural lesions on days 15 (Fig. 5c) and 21. Thereafter, partial restitution could be observed inducing a remarkable thickening of the germinative epithelium (Fig. 5d). Numerous necroses, however, and degenerating cells were still detectable in the seminiferous tubules on day 28, the local arrangement of spermatogenic cells in their different stage of development being still deeply disturbed in most tubules (Fig. 5d). Indications of recovery processes during the following days were not available as observations at later times were not made in the present experimental trial.

Discussion

From a morphological point of view, carboplatin is at least as toxic to the testicular tissue as cisplatin. This is the disappointing conclusion that can be drawn from the results of the present comparative study. Following treatment with carboplatin, early structural lesions of Sertoli cells occur and are first manifest by the disruption of their tight contacts to neighbouring cells, which are believed to be the structural correlate of the blood/testis barrier. Obviously, this effect is more pronounced in the case of carboplatin than with cisplatin. It is known that the integrity of the blood/testis barrier is an important prerequisite for the regular development and differentiation of spermatogenic cells. As consequence of the violation of this barrier, the processes of spermatogenesis are disturbed by the cytotoxic action of carboplatin in rather as they are with cisplatin, resulting in both compounds in the necrotization of spermatogenic cells being at diverse levels of differentiation. The numerous arrested mitotic figures appearing during spermatocytogenesis and both meiotic divisions following application of carboplatin are probably the consequence of molecular interactions between platinum-containing consecutive products of carboplatin and DNA molecules, which may be similar to those suggested for cisplatin [14, 22, 23]. This is confirmed biologically by the occurrence of analogous morphological features developing in the germinative epithelium following therapy with cisplatin and carboplatin and in normal and tumour cells treated with cisplatin [9, 10]. Additional deficiencies of cellular repair capacities in spermatogenic cells may be responsible for the comparably severe injuries of these cells, especially in view of the rather low concentrations of platinum found in the testes following treatment with cisplatin and carboplatin [11]. Besides impairing spermatocytogenesis and meiotic divisions, both compounds were also able to disturb the cellular differentiation processes of spermiohistogenesis. It is difficult to imagine that this effect could also be the consequence of a molecular interaction with DNA molecules, and this could be more easily explained by interference with other cellular molecules such as cytoplasmic proteins or cytoskeletal elements, effected by both compounds.

Carboplatin is one of the more effective second-generation antitumour platinum complexes that increasingly replace cisplatin in the clinical chemotherapy of tumours of the lung [2], the head and neck [4] and the ovary [1] and of testicular carcinomas [16, 27]. The main reason for this substitution is not the superior antitumour activity of carboplatin in comparison to cisplatin, but a different pattern of toxicity lacking nephrotoxicity, neurotoxicity and severe reduction of the general condition. The dose-limiting toxicity of carboplatin is caused by bone marrow suppression. This severely complicates combination chemotherapy with common organic cytostatics, which usually impair the bone marrow function, and leads to the paradoxical situation that chemotherapy with carboplatin can be carried out as effectively as with the parent compound cisplatin only when autologous bone marrow transplantation accompanies the chemotherapy [27]. Thus, some authors have councelled caution and declared that carboplatin should generally not be substituted for cisplatin [12, 13]. This caution is supported by the results of the present study, which show that testicular toxicity is not reduced following application of carboplatin, but that the structural lesions of Sertoli cells and spermatogenic cells are even worse and less reversible under the influence of carboplatin than with cisplatin, which was investigated in the present study at equitoxic dose levels under similar experimental conditions.

In this connection the basic question arises whether the disruption of the blood/testis barrier may even be a prerequisite for tremendous antitumour activity against testicular carcinomas. This seems to be confirmed by the obser-

vation that numerous other cytostatic agents, such as vincristine, procarbazine or busulfan [5, 8], similarly injure the testicular morphology. On the other hand, seen from a theoretical point of view, this condition is not thoroughly conclusive, as malignant testicular tumours destroy the blood/testis barrier by themselves as soon as they damage the Sertoli cells and penetrate the basal lamina surrounding the testicular tubules in the course of their invasive growth. At least then the malignant tumours should be vulnerable to cytotoxic agents even when these are not able by themselves to cross and to disturb the blood/testis barrier. The hope, therefore, remains that some of the antitumour platinum metal compounds of the third or fourth generation or some cytostatic non-platinum metal complexes might fulfil this condition not to destroy the intact blood/testis barrier of the remaining, healthy testis and, thus, may not induce long-lasting azoospermia and infertility following cytostatic chemotherapy.

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