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Does a gonadotropin-releasing hormone analogue prevent cisplatin-induced spermatogenic impairment?

An experimental study in the mouse

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Summary. The cytotoxic effect of *cis*-diamminedichloroplatinum II (cisplatin, CDDP) on spermatogenesis in BALB/C mice, and possible protection of the testes by leuprolide acetate (D-Leu-6 LHRH(1-9)-ethylamide, TAP-144, leuprolide), a synthetic gonadotropin-releasing hormone analogue, were examined. Temporary interruption of the pituitary-gonadal axis by the analogue and amelioration of the gonadal toxicity of cisplatin by reducing the cell division rate in spermatogenesis were expected. The results showed cisplatin to have a cytotoxic effect on spermatogenic cells in BALB/C mice. The administration of leuprolide had no effect on testicular weight or histological findings in the mouse testes. Pretreatment and simultaneous administration of leuprolide did not reduce the damaging effect of cisplatin on the testes in mice. This is at variance with a previously published report.

Key words: LH-RH analogue – Leuprolide acetate – Cisplatin – Mouse testis – Spermatogenesis

The recent development of chemotherapy regimens has improved the course of some malignancies even at an advanced stage. In recent years long-term survivors with Hodgkin's disease or acute lymphatic leukemia have become no longer rare. In the field of urology, current therapy of testicular tumors has led to an improved prognosis with an overall 5-year survival rate of more than 90% [5]. Since most of the survivors are of reproductive age, the effects of cancer therapy on fertility have taken on clinical importance.

Modern treatment of testicular neoplasms includes surgery, chemotherapy, and radiotherapy. Both chemotherapeutic agents and radiotherapy have the potential to depress or destroy spermatogenesis. The well-known impairment of spermatogenesis after chemotherapy of testicular cancer may also be attributable to the effect of cisplatin, which is used virtually all treatment regimens for testicular tumors, as well as vinblastine, bleomycin, etc. We investigated the effect of cisplatin on the dividing cells of seminiferous tubules of mouse testes and the role if an LH-RH analogue may play in preventing the damage. The rationale of this therapy is that an LH-RH analogue interrupts the pituitary-gonadal axis, reducing the rate of spermatogenesis, which may render the resting cells temporarily more resistant to cisplatin.

Materials and methods

Experiment 1: Effects of LH-RH analogue on cisplatin-induced testicular damage in mice

Eight-week-old male BALB/C mice were purchased from Charles River Co., Ltd., and maintained in a uniformly controlled room at 24 ± 2°C with 12 h light/12 h darkness, and provided with oriental mouse chow and water ad libitum.

The mice were divided into four groups of 5 or 6 males each and treated with 1.0 ml phosphate-buffered normal saline (PBS) given subcutaneously daily for 22 days (group I); 1.0 ml PBS containing 0.4 μg leuprolide acetate and 0.1% bovine serum albumin (BSA) as a carrier given subcutaneously daily for 22 days (group II); 1.0 ml PBS given subcutaneously daily for 22 days as in group I plus a single intraperitoneal injection of 5 mg/kg cisplatin on the 22nd day (group III); or given leuprolide subcutaneously daily as in group II plus a single intraperitoneal injection of 5 mg/kg cisplatin on the 22nd day (group IV).

One mouse in group II was accidentally lost. Twenty-nine days after cessation of treatment, two mice in each group were killed; the remaining animals were killed 56 days after treatment. The wet weights of the testes among the four groups were compared using Student's *t*-test. The testes were fixed in Bouin's fixative and stained with periodic acid-Schiff-hematoxylin (PAS) after routine paraffin embedding. Seminiferous tubules of each group were scored according to Johnsen's scoring method [10].

The mice were killed on the 29th and 56th day because it takes 29 days for types A1 through B spermatogonia to differentiate to the elongated spermatid stage. Spermatogenic cells which were already differentiating at the time of treatment would leave the tests within 56 days. Thus, 56 days is sufficient time to allow the surviving stem cells to progress through the differentiating pathway and produce a new generation of spermatozoa. This interval is also short enough to ensure that only minimal regeneration of stem cells occurs [13, 14].

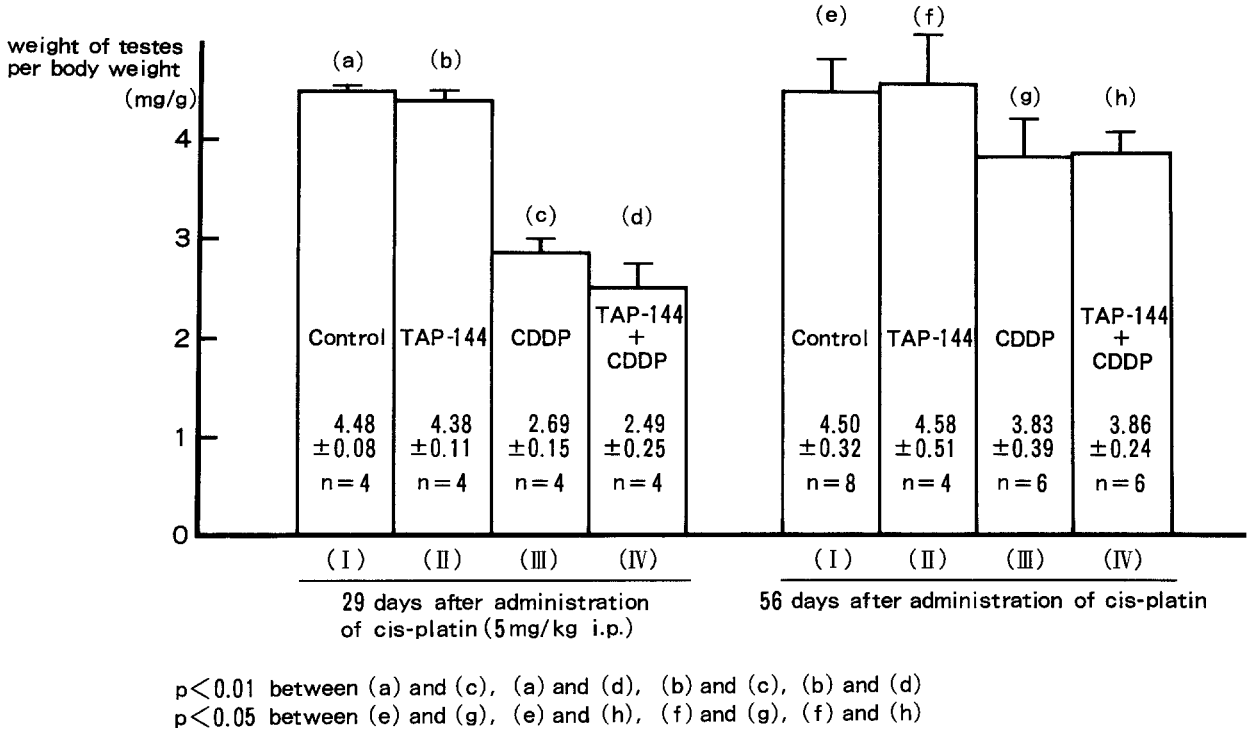

Fig. 1. Testicular per body weight of BALB/C mice after administration of cisplatin with or without leuprolide acetate (TAP-144)

Experiment 2: Effects of LH-RH analogue on the mouse testes

To reexamine the effects of leuprolide on the mouse testis, 8-week-old BALB/C mice were treated subcutaneously daily for 22 days with 1.0 ml PBS (group V); 1.0 ml PBS containing 1.0 μg leuprolide and 0.1% BSA (group VI); or 1.0 ml PBS containing 10.0 μg leuprolide and 0.1% BSA (group VII). The testes were weighed and histologically examined in these three groups as mentioned above in the same way as in experiment 1.

Results

Experiment 1

Testis weight was significantly reduced 29 days after cessation of the treatment in the mice to which cisplatin and cisplatin plus leuprolide had been administered (group III and IV respectively). The testicular weight loss had recovered to some degree by 56 days after treatment, although it remained significantly reduced in group III and IV compared with those of the control group (Fig. 1). On the 29th day, the number of spermatozoa and spermatids in the seminiferous tubules of the mice in groups III and IV was reduced, and testicular weight was also significantly lower than in the control group (Fig. 2; Table 1). However, there were no significant differences in testicular weight or histological findings between groups I and II or groups III and IV, either on the 29th or the 56th day (Fig. 1, 2; Table 1).

Experiment 2

No significant differences in testicular weight or histological findings were shown between any two of the three groups V, VI, and VII (Fig. 3, 4; Table 2). Irrespective of the administered dose, leuprolide did not influence the parameters under study to any extent.

Discussion

Due to marked recent progress in the chemotherapy of malignant disease, many patients with Hodgkin's disease, acute lymphatic leukemia, and testicular cancer can now be saved. Since the survivors are often of reproductive age, the infertility following successful cancer therapy has become of major concern. For example, MOPP chemotherapy (mustine, vinblastine, procarbazine, and prednisolone) has been estimated to result in azoospermia or severe oligozoospermia in at least 80% of male patients with Hodgkin's disease [16]. Brenner et al. [1] and Jewett and Tarvi [9] reported that most of the males patients who had undergone chemotherapy by the PVB (cisplatin, vinblastine, and bleomycin) or VAB VI (cyclophosphamide, vinblastine, actinomycin D, bleomycin, and cisplatin) regimens became azoospermic or severely oligozoospermic. In some cases, testicular function may recover, but it takes more than 2 years to recover from azoo- or oligozoospermia [9]. To minimize such persisting impairment of spermatogenesis, Glode et al. [7] proposed prior administration of leuprolide acetate, an LH-RH analogue. These authors suggested that the temporary inter-

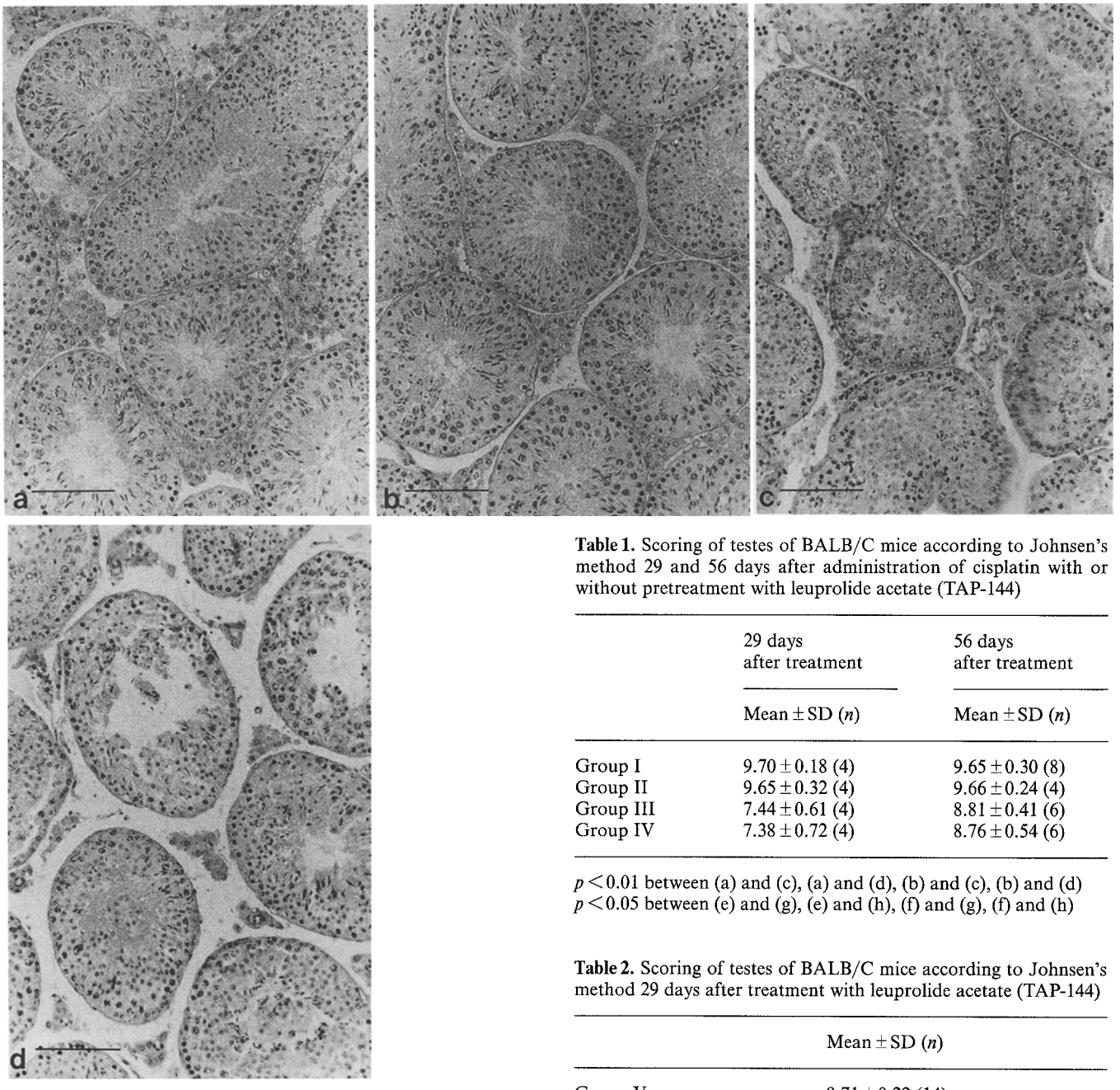

Fig. 2a–d. Histological findings in testes of mice in **a** group I, **b** group II, **c** group III, and **d** group IV, 29 days after administration of cisplatin with or without leuprolide acetate. Magnification ×180; *bars* represent 100 μm

Table 1. Scoring of testes of BALB/C mice according to Johnsen's method 29 and 56 days after administration of cisplatin with or without pretreatment with leuprolide acetate (TAP-144)

	29 days after treatment	56 days after treatment
	Mean ± SD (*n*)	Mean ± SD (*n*)
Group I	9.70 ± 0.18 (4)	9.65 ± 0.30 (8)
Group II	9.65 ± 0.32 (4)	9.66 ± 0.24 (4)
Group III	7.44 ± 0.61 (4)	8.81 ± 0.41 (6)
Group IV	7.38 ± 0.72 (4)	8.76 ± 0.54 (6)

$p < 0.01$ between (a) and (c), (a) and (d), (b) and (c), (b) and (d)
$p < 0.05$ between (e) and (g), (e) and (h), (f) and (g), (f) and (h)

Table 2. Scoring of testes of BALB/C mice according to Johnsen's method 29 days after treatment with leuprolide acetate (TAP-144)

	Mean ± SD (*n*)
Group V	9.71 ± 0.22 (14)
Group VI	9.66 ± 0.30 (14)
Group VII	9.72 ± 0.31 (14)

No significant differences in scores were found between any two groups

ruption of the pituitary-gonadal axis might reduce the rate of spermatogenesis and render the resting testis more resistant to the cytotoxic effects of chemotherapy. They demonstrated that temporary interruption of the pituitary-gonadal axis by an LH-RH analogue may lessen the gonadal toxicity of cyclophosphamide in respect of the weight and histology of testes in BALB/C mice.

LH-RH {(pyro)Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly NH)} consists of 10 amino acid residues [12]. The peptide bonds between Gly(6) and Leu(7), and between Pro(9) and Gly(10) are easily affected by peptidase, and the fragments derived from hydrolysis of LH-RH are biologically inactive. By replacement of Gly(6) and Gly(10) with another D-aminoacid and ethylamide, respectively, vari-

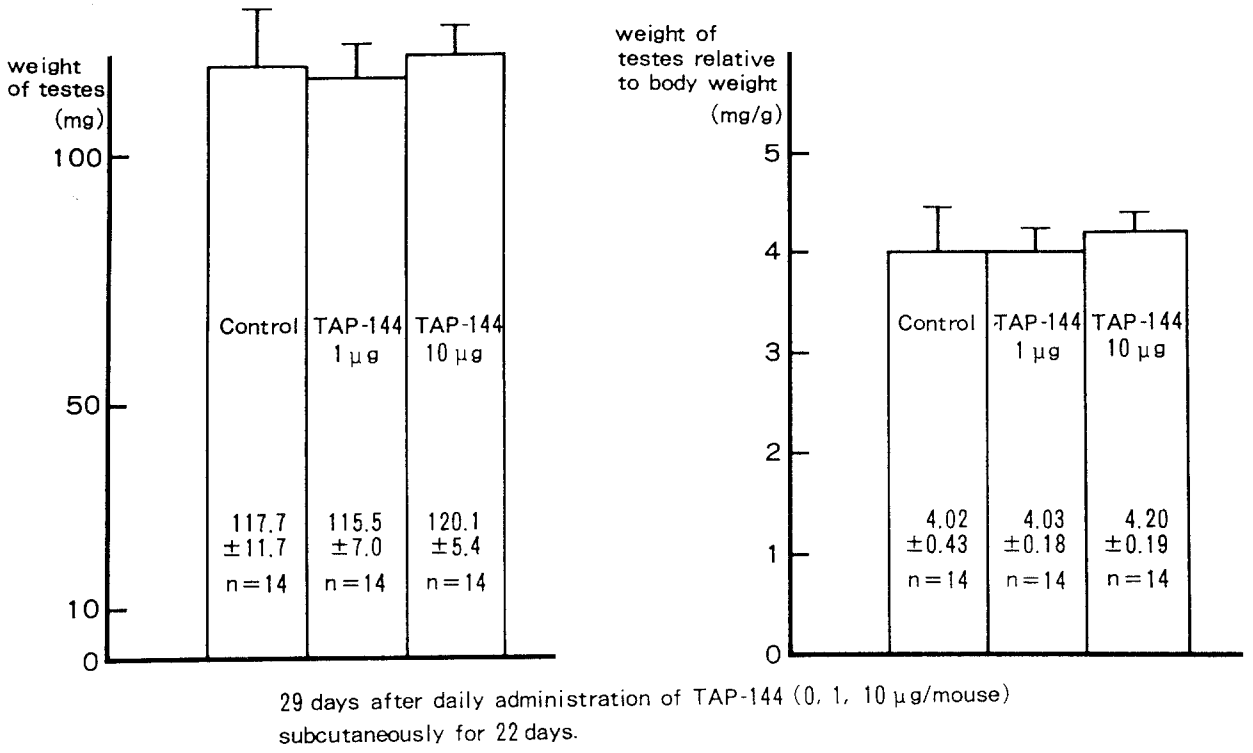

Fig. 3. Testicular weight and testicular weight relative to body weight in BALB/C mice 29 days after subcutaneous administration of leuprolide acetate (TAP-144) 0, 1, or 10 μg

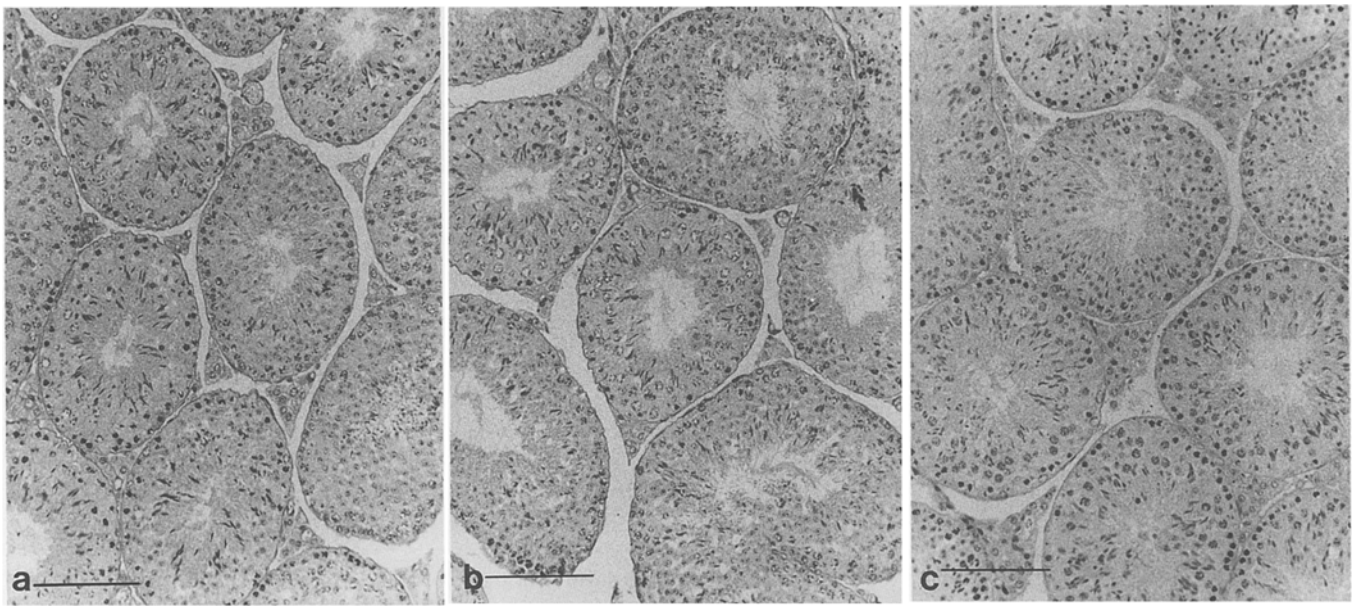

Fig. 4a–c. Histological findings in testes of mice in **a** group V, **b** group VI, and **c** group VII. Magnification ×180; *bars* represent 100 μm

ous LH-RH analogue have been developed and some of them have come into clinical use [3, 6, 11, 22]. Leuprolide as used in the experiments is one such compound.

The original incentive for the development of more potent LH-RH analogues was the expectation that the LH-releasing and ovulation-inducing effects of LH-RH observed in laboratory animals would be useful in the management of male and female infertility. As estimated from their LH-releasing activity, these synthesized LH-RH analogue are 100 times as potent as the natural LH-RH.

Physiological secretion of LH-RH is pulsatile, but continuous administration of natural LH-RH is well-known to cause suppression of LH secretion, mainly because of down regulation of the LH-RH receptor

(desensitization). The same phenomenon is also seen in prolonged administration of synthesized LH-RH analogues. Thus, LH-RH analogues are now commonly utilized to inhibit the secretion of gonadotropins and the gonadal sex hormones [24].

The chemical structure of LH-RH is identical in rodents and in humans [4, 15]. In the rat, the administration of LH-RH or LH-RH analogue causes a decrease in serum LH, testosterone, and in the number of LH receptors of Leydig cells [21]. These hormones have a striking inhibitory effect on dividing and proliferation spermatogenic cells [18].

Pretreatment and continued administration of leuprolide acetate has been reported to protect the testis against cyclophosphamide-induced damage in BALB/C mice [7]. According to the hypothesis by Glode et al. [7], dividing spermatogenic cells are liable to be affected by anticancer drugs, but decreased frequency of cell division or spermatogenic arrest in the seminiferous tubules induced by an LH-RH analogue reduces the sensitivity of spermatogenic cells to antineoplastic agents. Although the alkylating drugs may act on cells at any stage of the cell cycle, cytotoxity of cyclophosphamide is usually expressed when the cells enter the late G1 and S phase. The mechanism of action of cyclophosphamide is alkylation of the 7 nitrogen (N-7) of guanine residues in nucleic acids. Bifunctional alkylation by cyclophosphamide results in bridge formation between one N(7) and another N(7) of guanine residues of nucleic acid chains or between nucleic acid and protein by strong covalent bonds. This reaction causes the disruption in nucleic acid function [2].

The cytotoxic effects of cisplatin used in our present study are due to the reaction of hydrolyzed chloride atoms of cisplatin with DNA, forming intrastrand and interstrand cross-linkings. N(7) of guanine is very reactive and cross-links between adjacent guanines on the same or pairing DNAs are most readily demonstrated. Although the specificity of cisplatin to the phase of the cell cycle appears to differ with the cell types, the effects on cross-linking are most pronounced during the S phase. This cytotoxic action of cisplatin is similar to that of cyclophosphamide [2].

According to the hypothesis and the experimental results shown by Glode et al., it is necessary to begin treatment with an LH-RH analogue before chemotherapy, leading to spermatogenic arrest and to decreased cytotoxic effects on spermatogenesis by chemotherapeutic agents. However, the administration of leuprolide had no effect on testicular weight or the histology of the testes of BALB/C mice. In our study, unlike that of Glode et al., did not reduce the harmful effects of cisplatin on spermatogenesis in mice (Figs. 1, 2; Table 1).

A species difference exists in the sensitivity of testis to pituitary-mediated and direct antigonadal effects of LH-RH or its analogues [19]. LH-RH has rather different effects on the serum testosterone concentration is rats and in mice [20]. No reduction in serum testosterone levels or the number of the LH-receptors in Leydig cells has been observed in mice after administration of an LH-RH analogue, and mice are resistant to LH-mediated inhibitory effects on the testis [17, 23]. LH-RH analogues have been reported to have no direct effects on the mouse testis. By contrast, the rat testis is influenced by LH-RH analogue both directly and in a pituitary-mediated fashion [8].

Judging from these reports and our findings, we conclude that the administration of leuprolide acetate does not reduce the antispermatogenic effect of chemotherapeutic agents in mice. To determine whether LH-RH analogues protect human gonads under chemotherapy will require the design and conclusion of other studies.

Conclusion

Cisplatin had a severe by cytotoxic effect on spermatogenic cells in the testis of BALB/C mice, and the administration leuprolide, a potent LH-RH analogue, did not mitigate this toxicity. The administration of leuprolide had no influence on the mouse testis as suggested by recent reports [7].

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