Leuprolide Acetate Prevents Toxic Effects of Cisplatin on the Kidneys and Gastrointestinal Tract

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Abstract. We examined the protective effects of medical castration by means of gonadotropin-releasing hormone analogue (GnRH₄) on the toxic effects of cisplatin in rats. Twelve days after a s.c. injection of a slowly-releasable form of leuprolide acetate (GnRH₄SR), rats were injected i.p. with cisplatin daily (3 mg/kg body weight (BW) for males and 4 mg/kg BW for females) for four days and sacrificed 24 h after the last injection. The doses caused acute tubular necrosis and gastrointestinal (GI) symptoms, i.e., diarrhea and fluid retention and bleeding in GI tract. GnRH₄SR pretreatment reduced serum urea nitrogen (SUN) and serum creatinine (SCre) increase and the incidence of GI symptoms. Histological analysis showed that rats pretreated with GnRH₄SR had noticeably less kidney damage. GnRH₄ thus demonstrated its ability to protect the kidneys and GI tract against cisplatin toxicity in both male and female rats. This finding suggests a potential clinical application of GnRH₄ in antineoplastic chemotherapy.

Key words: GnRH, LH, Renotropic activity, Acute renal failure, Diarrhea

LONG-TERM treatment with gonadotropin-releasing hormone analogue (GnRH₄) has been shown to induce transient hypogonadism and inhibit the growth of sex-hormone-dependent tumors, including prostatic, breast, endometrial and ovarian cancers [1, 2]. Castration rendered the kidneys resistant to the nephrotoxicity of mercuric chloride in male rats [3]. Cisplatin, a heavy-metal compound like mercuric chloride, causes necrosis of the proximal tubules [4]. Therefore, to assess whether medical castration reduced the nephrotoxic activity of cisplatin, we studied the functional and morphological effects of a slow-release leuprolide acetate (GnRH₄SR) on cisplatin-induced renal damage. We also studied the effect of GnRH₄ on some of the other unpleasant, dangerous side effects that cisplatin has on the GI tract, such as emesis and intestinal damage [5, 6].

Materials and Methods

Fischer 344 rats, purchased from Charles River Japan, Atsugi city, were fed with a commercial rat chow and received tap water ad libitum. Microcapsules containing leuprolide acetate (TAP-144SR®, Takeda Pharmaceutical Co., Osaka; hereinafter GnRH₄SR) were injected s.c. at a dose of 0.47 mg for slow release over four weeks to induce hypogonadism [7]. Microcapsules without leuprolide acetate were injected as a placebo. Cisplatin, provided by Nippon Kayaku Co., Ltd., Tokyo, was dissolved with 0.9% saline immediately before use and was injected i.p. daily for four days. Diarrhea was recognized by dirty haunches.
in the animals treated. The rats were decapitated 24 h after the last cisplatin injection. The abdominal cavity was exposed to inspect the GI tract. SUN and SCre were measured as functional markers of renal damage. The kidneys were fixed in 10% formaline solution. After embedding in paraffin, sections were stained with hematoxylin and eosin. The extent of renal damage was graded by histological analysis. Plasma concentrations of LH and testosterone were measured by specific RIAs as described previously [8]. Plasma estradiol was measured with a commercial RIA kit (Daiichi Radioisotope Labs., Ltd., Tokyo). C.V. of intraassay variance were 3.3% at 3.7 pM and 3.2% at 14.7 pM. C.V. of interassay variance were 7.5% at 3.7 pM and 3.5% at 14.7 pM. Data are shown as the mean ± SEM. The analysis of variance and Duncan’s new multiple-range test were used to determine the significance of difference. Fischer’s exact probability test was used to determine the difference in incidence of GI symptoms.

Results

Dose-responsiveness of cisplatin toxicity

Seven-week-old male rats were given cisplatin daily over four days at the several doses shown in Table 1. Body weight decreased dose-responsive-ly. The highest dose (4 mg/kg BW) was sublethal, i.e., only two of the six treated survived. At a dose of 3 mg/kg BW, all rats survived but had significantly high SUN and SCre levels and a high incidence of diarrhea. Subsequent experiments with male rats were conducted at this dose. Six-week-old female rats were more resistant to cisplatin toxicity than their male counterparts, and SUN and SCre increase and the incidence of diarrhea was observed only at the highest dose, 4 mg/kg BW (Table 1). Subsequent experiments with female rats were conducted at this dose.

Effect of GnRH₄SR pretreatment on cisplatin toxicity in male rats

Twelve days after GnRH₄SR injection, the LH and testosterone plasma level and testicular weight decreased (Fig. 1 and Table 2). Cisplatin was then injected, significantly raising SUN and SCre levels in placebo- but not in GnRH₄SR-pretreated male rats (Fig. 1). Cisplatin-induced GI symptoms, i.e., diarrhea, fluid retention, and bleeding, were observed in all placebo-pretreated rats but not in any GnRH₄SR-pretreated rats (Table 2). Fluid retention exaggerated actual body weight, leading to an underestimation of the difference between the two groups of rats in body weight reduction caused by cisplatin. Renal damage was graded histologically (Table 3). Rats receiving cisplatin demonstrated necrosis, degeneration, and chromatin margination of the nucleus in the tubular epithelium of straight portions of proximal tubules (P₃) in the outer stripe of the outer medulla. Hyaline casts were also ob-

Table 1. Cisplatin dose responsiveness in rats

<table>
<thead>
<tr>
<th>Cisplatin dose (mg/kg/BW/day)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survivors/treated</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
<td>6/6</td>
<td>2/5</td>
</tr>
<tr>
<td>Body weight (g)b</td>
<td>205.6 ± 3.1</td>
<td>193.2 ± 0.6</td>
<td>186.8 ± 4.4</td>
<td>166.0 ± 3.5</td>
<td>167.5 ± 8.5</td>
</tr>
<tr>
<td>Diarrheaa</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>SUN (mg/dl)</td>
<td>23.1 ± 1.1</td>
<td>17.9 ± 0.7</td>
<td>28.6 ± 6.1</td>
<td>111.1 ± 35.5</td>
<td>61.1 ± 22.9</td>
</tr>
<tr>
<td>SCre (mg/dl)</td>
<td>0.36 ± 0.02</td>
<td>0.38 ± 0.02</td>
<td>0.58 ± 0.11</td>
<td>1.95 ± 0.60</td>
<td>0.85 ± 0.25</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survivors/treated</td>
<td>4/4</td>
<td>4/4</td>
<td>4/4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (g)b</td>
<td>133.8 ± 3.9</td>
<td>116.5 ± 2.1</td>
<td>116.0 ± 2.0</td>
<td>102.0 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>Diarrheaa</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>SUN (mg/dl)</td>
<td>21.1 ± 1.1</td>
<td>21.8 ± 4.2</td>
<td>23.3 ± 4.5</td>
<td>122.4 ± 19.4</td>
<td></td>
</tr>
<tr>
<td>SCre (mg/dl)</td>
<td>0.38 ± 0.03</td>
<td>0.40 ± 0.07</td>
<td>0.40 ± 0.04</td>
<td>1.40 ± 0.25</td>
<td></td>
</tr>
</tbody>
</table>

Rats were injected i.p. with cisplatin daily for four days and sacrificed 24 h after the last injection.

* Two of five rats treated survived. None died in other groups. b Body weight at sacrifice. c Numbers with diarrhea.
served in distal and collecting tubules, which were findings observed in acute tubular necrosis. Cisplatin damaged tubules so extensively that histological differences between GnRH₄ASR- and placebo-pretreated male rats were not so pronounced. GnRH₄ASR pretreatment did, however, lessen some of these morphological changes, especially hyaline casts (Table 3).
Effect of GnRH \(_{A}\) SR pretreatment on cisplatin toxicity in female rats

GnRH \(_{A}\) SR reduced the plasma estradiol level to one third of that in placebo-pretreated rats, although the difference was not statistically significant (Fig. 2). Ovarial weight decreased significantly (Table 2), indicating GnRH \(_{A}\) SR-induced medical castration. When cisplatin was administered, the reduction in body weight and the incidence of GI symptoms were significantly less marked in GnRH \(_{A}\) SR- than placebo-pretreated rats (Table 2). With GnRH \(_{A}\) SR pretreatment, cisplatin did not raise the SUN or SCr level (Fig. 2). Cisplatin-induced renal damage was histologically less marked in females than in males, and was lessened or prevented by GnRH \(_{A}\) SR pretreatment (Table 3). Representative figures are shown in Fig. 3.

Discussion

It was reported that male rats are more sensitive to nephrotoxicity of mercury [3] and chloroform [9] than females. This higher sensitivity in male rats was canceled by castration and was restored by supplementation with testosterone [3]. This sex-related difference was therefore reported to be attributable to the presence of testosterone in plasma, and was also observed herein on the nephrotoxicity of cisplatin. Interestingly enough, female rats also showed resistance to cisplatin toxicity in the GI tract. We aimed to examine whether reversible hypogonadism, induced by GnRH \(_{A}\) SR, can increase resistance to cisplatin toxicity. In rats treated with GnRH \(_{A}\) SR the weight of sex-hormone-dependent organs (prostates and ovaries) was reduced, indicating that these rats were in a state of hypogonadism for significant periods prior to sacrifice. A single s.c. injection of GnRH \(_{A}\) SR lessened or prevented the toxic activities of cisplatin both on kidneys (acute renal failure) and on the GI tract (diarrhea and fluid retention with bleeding) in both male and female rats. Despite a larger cisplatin dose, renal damage was histologically so less marked in female rats as to be prevented by GnRH \(_{A}\) SR pretreatment.

In male rats, castration increased the renal content of the sulfhydryl groups and rendered the kidneys resistant to heavy-metal-induced acute tubular necrosis [3], an effect attributable at least partially to a decrease in plasma testosterone in male rats. In female rats, estradiol reportedly protected against mercury-induced renal damage [10] but not against chloroform-induced damage [9].
The effects of female sex hormone on nephrotoxic agents are therefore contradictory. We did not aim to study whether ovarian steroids (estradiol and also progesterone) affect sensitivity to cisplatin toxicity, which remains to be clarified in a future study. In addition to the effects on gonadal steroids, long-term GnRH₄ treatment reportedly changed the biological activity and physicochemical properties of circulating LH [11], and had a direct inhibitory effect on the testis in reducing testosterone secretion in male rats [12]. These findings may explain the fact that plasma testosterone was decreased prominently even though plasma LH was decreased only slightly. We previously reported that some LH isoforms could stimulate the proliferation of renal proximal tubular cells [13–15]; this renotropically active isoform could improve renal function in subtotally nephrectomized castrated rats as an animal model of chronic renal failure [16]. It is physiologically significant that the renotropic activity of LH was exhibited in castrated animals and inhibited by testosterone [17]. Decreased plasma testosterone thus activated the renotropic activity of LH. Although GnRH₄SR was decreased plasma LH, a significant amount of LH was still present in plasma. This plasma LH under the very low levels of testosterone may possibly render the kidneys resistant to cisplatin, but, an involvement of the mechanism, not mediated by endocrinological changes, could not be excluded in this study.

GnRH₄SR prevented unwanted GI symptoms in both males and females. This finding may have clinical implications for patients suffering the unpleasant, dangerous side effects of cisplatin treatment. The precise nature of this protective mechanism as it relates to the kidneys and GI tract remains to be clarified, however.

A loss of body weight was associated with the induction of acute tubular necrosis and GI tract impairment. GnRH₄ pretreatment reduced the loss in body weight in female rats and probably in male rats, also, additional evidence indicating the potentially favorable effects of GnRH₄.

GnRH₄ is currently widely used to inhibit the growth of sex-hormone-dependent neoplasms without significant adverse side effects [1, 2]. In addition to this anti-neoplastic activity, GnRH₄ pretreatment also protects against chemotherapy-induced ovarian follicular loss [18] and testis damage [19]. Our current findings thus add to the potentially favorable clinical applications of GnRH₄.

Attempts have been made to ameliorate the nephrotoxic activity of cisplatin, e.g., by hydration in clinical studies and by bismuth-induced renal accumulation of metallothionein in experimental studies [20]. Cisplatin-induced GI symptoms can also be reduced by a selective serotonin subtype 3 (5HT₃) receptor antagonist, ondansetron [5]. GnRH₄ has advantages over these agents because it simultaneously prevents the two major toxicity problems and provides antineoplastic activity in some neoplasms following only a single injection.
Fig. 3. Effects of GnRH₄SR pretreatment on renal damage in female rats treated with cisplatin. Prior to cisplatin injection, the rats were injected with either a placebo (a and c) or GnRH₄SR (b and d). a: Arrows indicate necrotic areas in the pars recta of proximal tubules (P₃) in the outer stripe of the outer medulla. (×40); b: no necrosis was observed in rats pretreated with GnRH₄SR. (×40); c: increased magnification (×400) of a. Necrosis (thick medium-length arrows), degeneration (short thick arrows), nuclear chromatin margination (long thin arrows), and hyaline cast (large arrow); d: increased magnification (×400) of b. No necrosis was observed. Less extensive degeneration (short thick arrows) and nuclear chromatin margination (long thin arrows) of tubular epithelium in P₃.
To summarize, GnRH₃SR pretreatment can prevent or ameliorate the toxic effects of cisplatin on the kidneys and GI tract in both male and female rats. Given the limitations such toxicity presents in clinical work, the protective effect of GnRH₃SR suggested a potentially important possibility in clinical applications.

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References