Endocrinological Studies on TAP-144-SR, a Sustained-Release Formulation of a Potent GnRH Agonist (D-Leu⁶-[des-Gly¹⁰-NH₂]-GnRH Ethylamide), in Male Rats

KATSUICHI SUDO* TSUNEO MASAKI, KUNIO SHIOTA#,
MASAHIRO KAWASE AND TAKESHI FUJITA

*Biology Research Laboratories, Research and Development Division,
Takeda Chemical Industries, Ltd.
17-85, Juso-Honmachi 2-Chome, Yodogawa-ku, Osaka 532, Japan*

Abstract

The paradoxical effects of TAP-144-SR, a biodegradable sustained-release formulation of a potent GnRH agonist (TAP-144, leuprolide acetate) were evaluated in male rats by comparing its potency with that of TAP-144 solution.

A single sc injection of TAP-144-SR (equivalent to 0.1 mg/kg/day as TAP-144), prepared by encapsulating the agonist in microcapsules of copoly (DL-lactic/glycolic acid), suppressed serum levels of androgens, and the levels remained suppressed for 4 weeks.

The potency of the paradoxical effects of TAP-144-SR was evaluated 4 weeks after treatment by comparing it with that of TAP-144 solution administered daily for 4 weeks. Both daily injections of TAP-144 solution and a single injection of TAP-144-SR (equivalent to 0.02, 0.2 or 2 mg/kg/day as TAP-144) decreased the weight of the testes, prostates and seminal vesicles in a dose-dependent manner in a 4-week assay in male rats. TAP-144-SR was more effective than TAP-144 solution in reducing these organ weights. Serum and pituitary concentrations of LH and FSH and serum testosterone levels were also lower in TAP-144-SR-treated than in TAP-144 solution-treated rats. These results indicate that the paradoxical effects were more extensive upon TAP-144-SR treatment, suggesting that maintaining constant serum TAP-144 levels results in more extensive desensitization of the pituitary and testes. These results also suggest advantages of TAP-144-SR over TAP-144 solution in both efficacy and convenience as an anti-prostatic tumor agent.

Potent GnRH agonists both stimulate and inhibit gonadal function depending on the dose and duration of treatment in man

and laboratory animals (Auclair *et al.*, 1978; Rivier *et al.*, 1979; Bhasin & Swerdloff, 1986). The inhibition of gonadal function caused by chronic treatment with these potent GnRH agonists, i.e. paradoxical effects, has been clinically utilized to induce medical castration for treating a variety of hormonally responsive clinical disorders (Warner *et al.*, 1983; Yen, 1983;

Received April 11, 1990

#: The present address is Department of Agriculture, University of Tokyo, Bunkyo-ku, Tokyo 113.

*: To whom correspondence should be addressed.

Nicholson *et al.*, 1984). However, the necessity of daily injection was an obstacle to broader and more practical use of the agonists. Currently, nasal (Huhtaniemi *et al.*, 1985; Reznik *et al.*, 1987) and sustained-release (SR) formulations (Redding *et al.*, 1984; Walker *et al.*, 1986) are being tried or practically used to overcome the problem of daily injection of GnRH agonists. A biodegradable injectable SR formulation of ICI 118630, a small cylindrical rod consisting of copoly (DL-lactic/glycolic acid) (PLGA) copolymer, was developed and has been reported to be effective in reducing serum testosterone in prostate cancer patients (Walker *et al.*, 1984).

Recently, biodegradable microcapsules of PLGA containing a potent GnRH agonist, TAP-144 (D-Leu⁶-[des-Gly¹⁰-NH₂]-GnRH ethylamide acetate, leuprolide acetate; Fujino *et al.*, 1974; Rippel *et al.*, 1975), were developed to maintain a constant release for 1 month (Ogawa *et al.*, 1988a, b). The administration of TAP-144-SR has been shown to decrease testosterone levels and prostate weight in male rats and dogs (Ogawa *et al.*, 1989; Okada *et al.*, 1989) and to decrease serum estradiol levels in female rats (Okada *et al.*, 1988). Besides the advantage of reducing the frequency of injection, maintaining constant TAP-144 levels in serum by TAP-144-SR administration may modify the paradoxical effects of the GnRH agonist.

The present study was undertaken to evaluate TAP-144-SR by comparing its endocrinological effects, especially the paradoxical effects, with those of TAP-144 solution.

Materials and Methods

Animals and treatment

TAP-144-SR was prepared by suspending TAP-144-containing microcapsules of PLGA copolymer in 2.5 ml/kg of a suspension vehicle.

The concentration of TAP-144 in the microcapsules was 8%. TAP-144 solution was prepared in 1 ml/kg of saline. The method of preparing TAP-144-containing microcapsules is described in detail elsewhere (Ogawa *et al.*, 1988a, b.) Doses of TAP-144-SR are expressed as the amount of TAP-144 contained in the microcapsules. The daily equivalent dose was calculated by dividing the injected dose by 30.

Male Sprague-Dawley rats at 10 weeks of age (Charles River Japan, Inc.) received either a daily sc injection of the solution or intermittent sc injections of TAP-144-SR. Rats in the control group received the suspension vehicle. Rats in the castration group were subjected to orchietomy on the 1st day of the treatment. Blood was weekly withdrawn from the tail vein of the same animals (Experiment 1, Figure 1) or from the abdominal aorta (Experiment 2, Figures 4 and 5) under ether anesthesia. Prostate weight was determined after combining both ventral and dorsal lobes.

Experiment 1: Rats were treated for 6 weeks with sc injections of TAP-144-SR (equivalent to 0.1 mg/kg/day as TAP-144) as follows.

1/6 W: treated at 0 week

1/4 W: treated at 0 and 4 weeks

1/2 W: treated at 0, 2 and 4 weeks

Experiment 2: Rats were treated for 4 weeks with either daily sc injections of TAP-144 solution or one injection of TAP-144-SR at 3 different doses.

Radioimmunoassay

Serum concentrations of LH and FSH were determined by a double antibody radioimmunoassay using NIADDK rat RIA kits (Sudo *et al.*, 1979). Purified LH (NIADDK-rat LH-I-7) and FSH (NIADDK-rat FSH-I-4) were labeled with ¹²⁵I(¹²⁵I-NaI, New England Nuclear, Du Pont) according to the chloramine-T method of Greenwood *et al.* (1963). Serum levels of LH and FSH are expressed in terms of NIAMDD-rat LH-RP-1 and NIAMDD-rat FSH-RP-1, respectively. Pituitary concentrations of LH and FSH were determined after homogenization of pituitary in 50 mM Tris-boric acid buffer containing 5 mM EDTA at pH 7.9 and centrifugation at 1,000 g × 20 min. The serum testosterone concentration was determined with a radioimmunoassay kit purchased from Green Cross, Ltd. Serum levels

of androgens were determined with a rabbit antiserum to testosterone-BSA conjugate which cross-reacts with testosterone and 5 α -dihydrotestosterone to the same extent (in preparation). Radioactive testosterone (925 GBq (25 Ci)/mmol, 7- 3 H(N), New England Nuclear, Du Pont)

and unlabeled testosterone (Sigma) were used in radioimmunoassay for serum androgens.

Statistical analysis

Statistical analysis was carried out by analysis of variance followed by Dunnett's test.

Fig. 1. Serum androgen concentrations in rats intermittently treated with TAP-144-SR (equivalent to 0.1 mg/kg/day as TAP-144) for 6 weeks. 1/6W: treated at 0 week; 1/4W: treated at 0 and 4 weeks; 1/2W: treated at 0, 2 and 4 weeks. The value at 0 week is the mean of the control values from 1 to 6 weeks. Mean $\pm$ SEM (n=5 or 6). *: $P < 0.05$, **: $P < 0.01$ (compared with the control by analysis of variance followed by Dunnett's test)

Table 1. Organ weights in rats receiving sc injections of TAP-144-SR at different intervals. Rats were sc treated with TAP-144-SR (equivalent to 0.1 mg/kg/day as TAP-144) for 6 weeks.

	No. of rats	Body weight (g)	Testes (mg)	Prostates (mg)	Seminal vesicles (mg)
Control	5	500 $\pm$ 13	2687 $\pm$ 239	1013 $\pm$ 69	1307 $\pm$ 64
Castrated	6	467 $\pm$ 10		100 $\pm$ 6**	90 $\pm$ 2**
TAP-144-SR					
1/6W (0)#	6	493 $\pm$ 8	2144 $\pm$ 118	622 $\pm$ 79**	620 $\pm$ 132**
1/4W (0, 4W)	6	479 $\pm$ 14	2051 $\pm$ 156*	530 $\pm$ 58**	289 $\pm$ 59**
1/2W (0, 2, 4W)	6	487 $\pm$ 13	1954 $\pm$ 134*	448 $\pm$ 57**	274 $\pm$ 66**

#: Weeks when rats received treatment

Mean $\pm$ SEM. *: $P < 0.05$, **: $P < 0.01$ (compared with the control by analysis of variance followed by Dunnett's test)

Fig. 2. Serum and pituitary concentrations of LH and FSH in rats intermittently treated with TAP-144-SR (equivalent to 0.1 mg/kg/day as TAP-144) for 6 weeks.

Abbreviations are described in the legend to Figure 1.

Mean $\pm$ SEM (n=5 or 6). *: $P < 0.05$, **: $P < 0.01$ (compared with the control by analysis of variance followed by Dunnett's test)

Results

Effects of TAP-144-SR treatment at different intervals on serum levels of androgens and genital organ weights

As a preliminary study, the effects of TAP-144-SR were examined by changing intervals of administration. TAP-144-SR (equivalent to 0.1 mg/kg/day as TAP-144) was sc injected one, two or three times during the 6-week treatment period. As shown in Fig. 1, serum concentrations of androgen were decreased 1 week after the initiation of the treatment in all rats treated with TAP-144-SR as well as in castrated rats. The decreases in serum androgen in the 1/4 W and 1/2 W groups were essentially the same, and they were maintained until the end of the 6-week period. After the 6-week treatment, the weights of testes, seminal vesicles and prostates were significantly lower in treated rats than in control rats (Table 1). The decreases in the weights of these organs in the 1/6 W group were less marked than in other groups, 1/2 W and 1/4 W.

Serum and pituitary concentrations of LH and FSH in castrated rats were significantly higher than those in the control group (Fig. 2). In TAP-144-SR-treated rats, serum concentrations of LH remained unchanged and serum concentrations of FSH were significantly lower. Pituitary concentrations of LH and FSH were also significantly lower in treated rats. These decreases were more marked in the 1/4 W and 1/2 W groups than in the 1/6 W group.

Fig. 3. Organ weights of rats treated either daily with TAP-144 solution or once with TAP-144-SR (equivalent to 0.02, 0.2 or 2 mg/kg/day as TAP-144). Rats were sc treated for 4 weeks. Mean $\pm$ SEM (n=5 or 6). *: $P < 0.05$, **: $P < 0.01$ (compared with the control by analysis of variance followed by Dunnett's test)

Comparison of the endocrinological activities of the two formulations, TAP-144-SR and TAP-144 solution

Administration of TAP-144 (0.02, 0.2 or 2 mg/kg/day) as either daily injections of TAP-144 solution for 4 weeks or as an equivalent single injection of TAP-144-SR dose-dependently suppressed the weights of accessory sex organs and the testes (Fig. 3). The organ weight decreasing effects were more marked in the SR-treated rats.

The weight of the pituitary remained unchanged.

In castrated rats, serum concentrations of LH and FSH were significantly higher than they were in the control rats (Fig. 4). In TAP-144 solution-treated rats, serum LH remained unchanged, serum FSH increased slightly at the two lower doses and pituitary concentrations of LH and FSH decreased. In TAP-144-SR-treated rats, serum LH remained unchanged, serum FSH

Fig. 4. Serum and pituitary concentrations of LH and FSH in rats treated either daily with TAP-144 solution or once with TAP-144-SR (equivalent to 0.02, 0.2 or 2 mg/kg/day as TAP-144). Rats were sc treated for 4 weeks. Mean $\pm$ SEM (n=6). *: $P < 0.05$, **: $P < 0.01$ (compared with the control by analysis of variance followed by Dunnett's test)

Fig. 5. Serum concentrations of testosterone in rats treated either daily with TAP-144 solution or once with TAP-144-SR (equivalent to 0.02, 0.2 or 2 mg/kg/day as TAP-144). Rats were sc treated for 4 weeks. Mean $\pm$ SEM (n=6). *: $P < 0.05$, **: $P < 0.01$ (compared with the control by analysis of variance followed by Dunnett's test)

decreased and pituitary concentrations of LH and FSH decreased.

The serum concentration of testosterone decreased in rats castrated or treated with either of the two TAP-144 formulations (Fig. 5). In TAP-144-SR-treated rats, the testosterone levels were slightly lower than those in the rats treated with TAP-144 solution.

Discussion

In the present study, the paradoxical effects of a potent GnRH agonists were evaluated by maintaining constant levels of the agonist for 4 weeks with a sustained-release formulation, TAP-144-SR (Ogawa *et al.*, 1989; Okada *et al.*, 1989). The results of this study showed that a single sc injection of TAP-144-SR suppressed serum

concentrations of androgens for 4 weeks in male rats. These results are in a good accordance with those previously reported (Ogawa *et al.*, 1989; Okada *et al.*, 1989). The present study also showed that reduction in the weight of accessory sex organs was more marked in TAP-144-SR than with TAP-144 solution treatment when comparison was made based on daily equivalent doses. TAP-144-SR is 2–6 times as potent as TAP-144 solution in reducing serum testosterone levels. Pituitary concentrations of LH and FSH were also suppressed more markedly in TAP-144-SR-treated rats. The higher efficiency of TAP-144-SR as compared to TAP-144 solution coincides with the pharmacological effects in rat prostate cancer (Ichikawa *et al.*, 1988) and experimental endometriosis (Okada *et al.*, 1988). A few other potent GnRH agonists have been reported to cause a more marked reduction in testosterone levels in rats and humans when they are administered as an SR formulation as opposed to a solution (Akhta *et al.* 1983; Redding *et al.*, 1984; Walker *et al.*, 1984). The difference in potency between the SR and solution formulations of TAP-144 is roughly equal to that for ICI 118630 (Walker *et al.*, 1984).

The paradoxical effects of GnRH agonists are thought to be mainly due to desensitization of the pituitary and gonads (Bhasin & Swerdloff, 1986). Therefore, the results of the present study suggest that maintaining constant concentrations of GnRH agonists induces more extensive desensitization than does repeated daily injection.

Desensitization of the gonads to gonadotropins is regarded as an important mechanism for the reduction in the sex hormone concentration caused by GnRH agonists (Auclair *et al.*, 1978; Tcholakian *et al.*, 1978; Fraser and Baird, 1987). Desensitization of the gonads is often associated with high serum gonadotropin concentrations (Bhasin & Swerdloff, 1986). Although serum LH concentrations in TAP-

144-SR-treated rats are not higher than those in the control rats, the desensitization might have been induced by high serum concentrations of LH shortly after the TAP-144-SR treatment (submitted for publication). Additionally, the desensitization may continue as long as serum TAP-144 concentrations are maintained at levels sufficient to stimulate the pituitary. In fact, TAP-144-SR-treated rats produce very little testosterone upon hCG treatment (submitted for publication). Also, other factors such as a decrease in bioactive gonadotropins and/or continued direct inhibitory action on the gonads (Hsue and Jones, 1981; Cooke and Sullivan, 1985; Bhasin & Swerdloff, 1986) may contribute to a decrease in testosterone levels following TAP-144-SR treatment.

Extreme reductions in the pituitary concentrations of LH and FSH in TAP-144 treated rats receiving either the sustained-release or the solution form, may have some relationship to the desensitization of the pituitary after treatment with GnRH agonists (Bhasin & Swerdloff, 1986). Exhaustion of pituitary LH and FSH due to continuous stimulation by TAP-144 may be partially responsible for the complete loss of response; serum LH and FSH levels in TAP-144-SR-treated rats were no longer higher than those in the control rats, despite the presence of TAP-144 in the serum.

The results of the present study suggest that TAP-144-SR, as opposed to TAP-144 solution, has the therapeutic advantages of reducing the frequency of injection and enhancing the pharmacological activity.

Acknowledgement

We thank Mr. Y. Yoshida and Miss Y. Akinaga for their technical support. We also thank NIADDK (NIH) for the gift of pituitary hormone assay kits.

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