J. Steroid Biochem. Molec. Biol. Vol. 37, No. 6, pp. 937–942, 1990
Printed in Great Britain.

0960-0760/90 $3.00 + 0.00

EFFECT OF THE NEW POTENT LHRH ANTAGONIST ANTIDE

URSULA-F. HABENICHT,* MARTIN R. SCHNEIDER and M. FATHY EL ETREBY
Research Laboratories of Schering AG, Berlin, F.R.G.

Summary—The ability of the new LHRH antagonist antide to induce a long-term chemical castration in adult male rats and cynomolgus monkeys was investigated. The animals were treated subcutaneously with different doses either once or on 5 consecutive days. The effects on serum concentration of LH (only rat) and testosterone and on the weights of the testes, prostates and seminal vesicles were investigated after different periods of time. Histological evaluation of testes, pituitary and hypothalamus was also performed. In the rat a clear dose-dependent inhibitory effect on the above mentioned parameters was observed whereby long-lasting castration-like effects were achieved at concentrations between 6 ($\leqslant$8 weeks) and 15 mg/kg (>8 weeks). In the cynomolgus monkey a prolonged inhibitory effect was induced only at 15 mg/kg and the duration was only 2–3 weeks. Histologically, no signs indicative of irreversible effects were observed in either species. In conclusion: although species differences became evident in terms of the duration of a long-lasting inhibitory effect on the male reproductive system, antide exhibited such an effect in the rat and the monkey and was able to induce a chemical castration in both species. In addition, using the rat Dunning R 3327 prostatic carcinoma model, 10 mg/kg antide given subcutaneously every 6 weeks for a total period of 26 weeks, had an inhibitory effect on tumor growth identical to that of castration emphasizing the suitability of this compound for treatment of prostatic cancer.

INTRODUCTION

LHRH agonists have now found their place in the armamentarium of drugs for treatment of prostatic cancer [1, 2]. Although this type of compound has been shown to be as effective as other types of androgen deprivation in terms of survival rate, one major drawback lies in the initial stimulation of the testosterone biosynthesis which can produce a painful flare of the disease, resulting in a profound worsening of the quality of life [3, 4]. In this context, LHRH antagonists might be of advantage in so far as an immediate inhibition of testosterone production can be induced [5–7]. However, the development of this type of compound has not been very promising until very recently due to an undesired histamine release as a side effect [8–10]. Now, new antagonists have been developed which are characterized by an improved potency and much lower histamine releasing potential than the earlier types [7, 8, 11–13]. The LHRH antagonist antide [*N*-Ac-D-Nal(2)^1, D-Pal(3)^3, Ser^4, Nic-Lys^5, D-Nic-Lys^6, Leu^7, I-Lys^8, Pro^9, D-Ala^{10}NH$_2$] synthesized by Folkers and Bowers belongs to this new type of compound [13].

An interesting aspect of the pharmacology of antide was described by Leal *et al.* [14], who showed that single doses of antide had a profound long-lasting inhibitory effect on serum LH concentration in castrated female cynomolgus monkeys. It has, however, been reported that castrated animals are more sensitive to an inhibitory effect in this respect than intact animals [7, 15, 16]. Since, in addition, species-specific effects might be anticipated, we wished to acertain whether such a long-lasting inhibitory effect could be induced in intact male rats as well as in intact male cynomolgus monkeys, resulting in an effective inhibition of the male reproductive system. In addition, the efficacy of antide was tested in a prostatic cancer model using the androgen-dependent Dunning R 3327 tumor of the Copenhagen rat.

EFFECTS IN INTACT MALE RATS

Intact adult male Han Wistar rats were treated subcutaneously with different doses of antide either once (1, 3, 6, 10 and 15 mg/kg) or

Proceedings of the 2nd International EORTC Symposium on "Hormonal Manipulation of Cancer: Peptides, Growth Factors and New (Anti-)Steroidal Agents", Rotterdam, The Netherlands, 9–11 April 1990.
*To whom correspondence should be addressed: Dr Ursula-F. Habenicht, Schering AG-Exp. Andrologie und Oncologie, Müllerstr. 170–178, D-1000 Berlin 65, F.R.G.

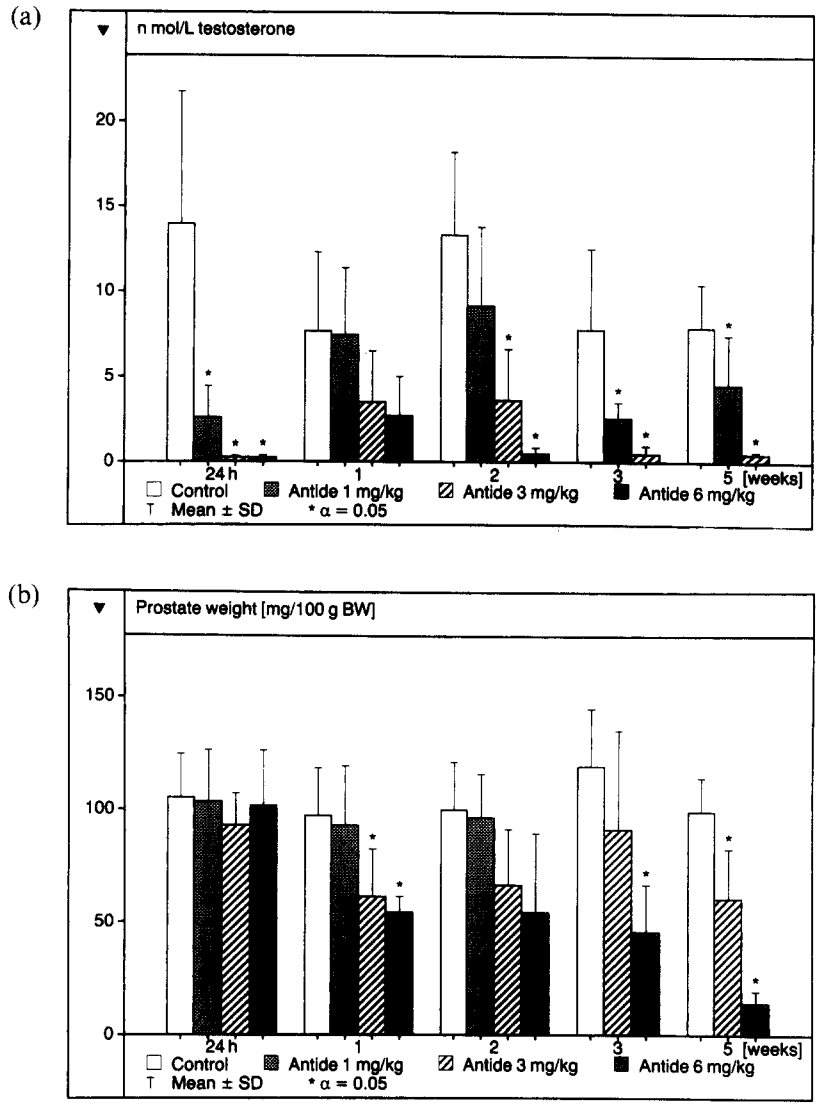

Fig. 1. The effect of a single subcutaneous injection of the LHRH antagonist antide on serum testosterone concentration (a) and on prostate weight (b) in intact adult male rats after different periods of time.

on 5 consecutive days (5 × 3 mg/kg). The effects on the concentration of serum testosterone and on the weights of the prostate, seminal vesicles and testes were investigated after different periods of time (24 h, 1, 2, 3, 5 and 8 weeks). A clear dose-dependent inhibitory effect on the above mentioned parameters was observed. The minimal dose to achieve a long-term inhibitory effect on testosterone production and a castration-like decrease in prostate weight was 6 mg/kg (Fig. 1a and b). However, 5 weeks were needed before prostate weights reached this degree of inhibition. The most effective treatment schedule was the single application of 15 mg/kg resulting in prostate weights at the castration level within 2 weeks and maintainance at this level 8 weeks after the injection. Hormone levels (serum testosterone concentration) showed that the phase of recovery had just begun at this time (Fig. 2a and b).

The histology of the testes, the pituitary and the hypothalamus did not show any evidence indicative of an irreversible effect. The degree of inhibition of spermatogenesis and of immunohistochemically detectable LHβ in the pituitary was in agreement with the stage of hormone deprivation.

Antide was well tolerated even at the highest doses. No signs for the induction of a histamine release were evident.

EFFECTS IN MALE CYNOMOLGUS MONKEYS

Intact adult male cynomolgus monkeys were treated subcutaneously either once

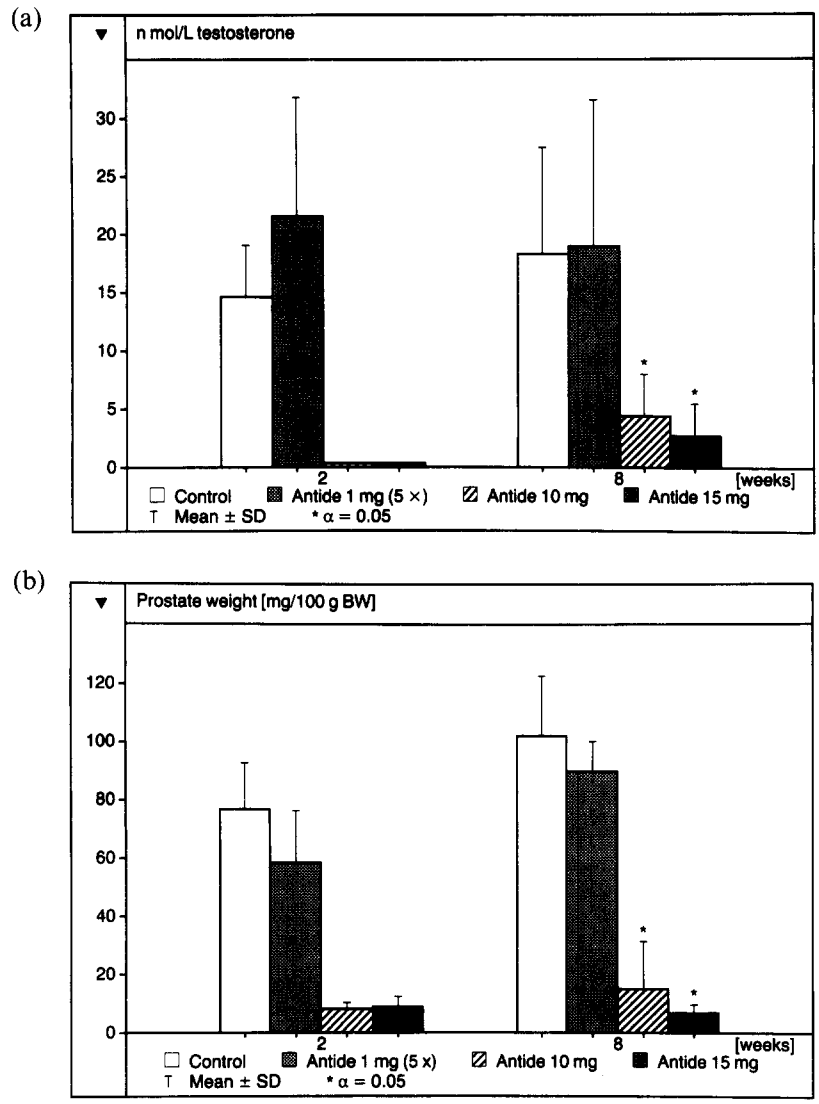

Fig. 2. Long-term effect of a single subcutaneous injection of the LHRH antagonist antide at high concentrations on serum testosterone values (a) and on prostate weights (b) in intact adult male rats after 2 and 8 weeks.

(6 mg/kg, 15 mg/kg) or on 5 consecutive days (5 × 3 mg/kg).

The effects on serum concentration of testosterone were investigated after different periods of time (24 h–15 weeks). In one experiment, 4 out of 8 antide-treated animals (5 × 3 mg/kg) were sacrificed after 4 weeks (3 weeks after the last injection)—at a time, when serum testosterone values were still depressed—and the remainder after 15 weeks. In contrast to the situation in the rat, it was not possible to induce a long-term inhibitory effect of the testosterone production with 6 mg/kg in this species. The values were depressed within 24 h but recovered within 1 week. A long-term inhibition, however, was achievable with 15 mg/kg—given either as 1 single injection, or with 3 mg/kg given on 5 consecutive days. The effect lasted for 2–3 weeks. The phase of recovery was characterized by an extremely pronounced rebound phenomenon. The testosterone values returned to normal by the end of the observation period (Fig. 3).

The histology of the prostate of the animals treated with multiple injections (5 × 3 mg/kg) and sacrificed 4 weeks after the first injection showed a severe atrophy of the acinar structures (Fig. 4a). On the other hand, the prostates of the remaining animals sacrificed after 15 weeks were characterized histologically by a high secretory activity (Fig. 4b), indicating the reversibility of the inhibitory effects at the level of this target organ. In addition, no signs indicative of irreversible effects were observed in the testes.

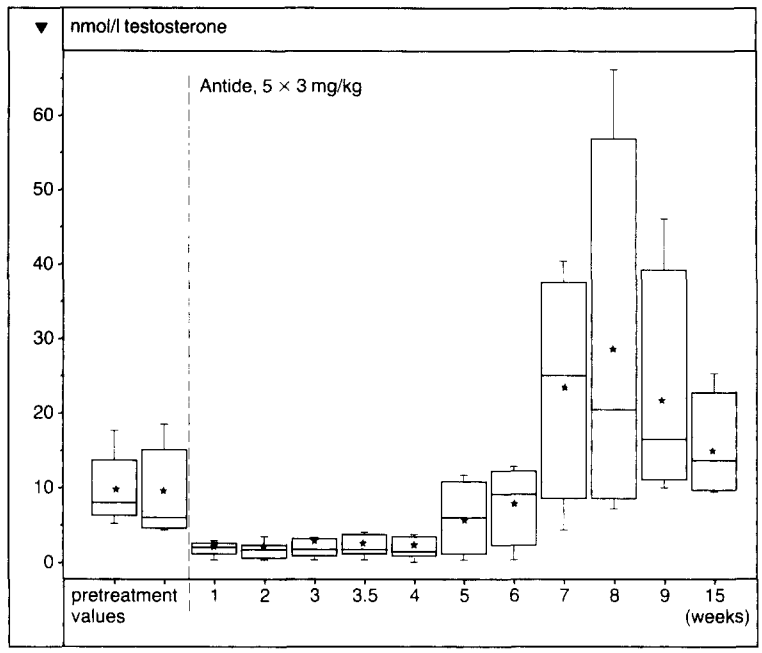

Fig. 3. Long-term effects of multiple injections of the LHRH antagonist antide on serum testosterone concentration in intact adult male cynomolgus monkeys.

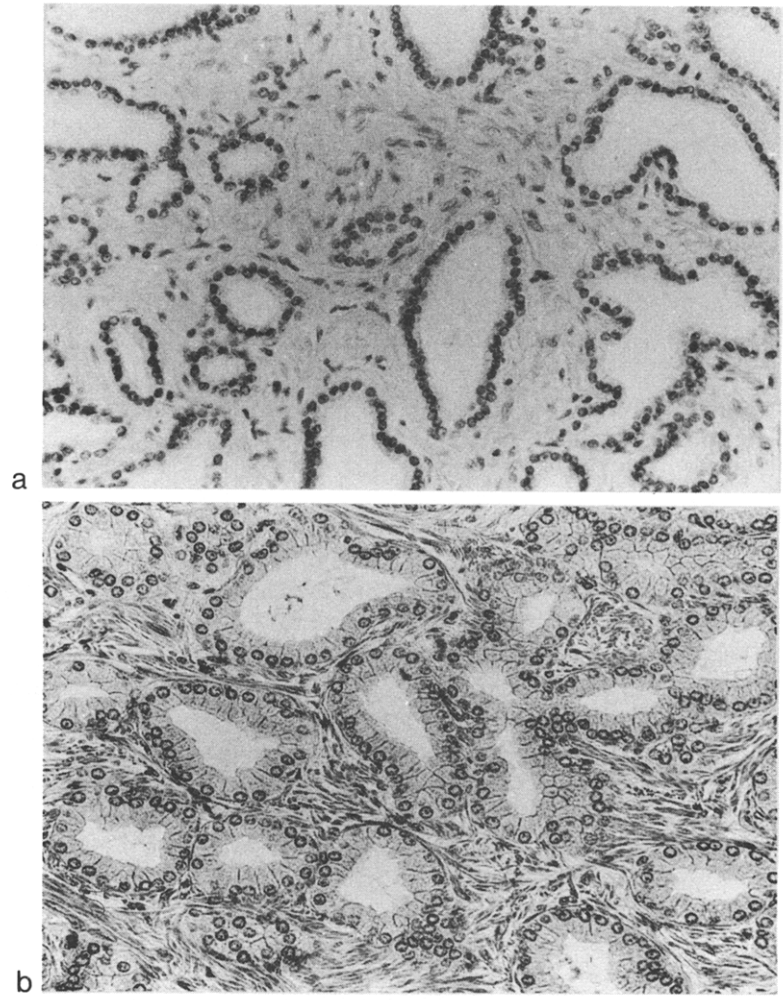

Fig. 4. Histology of the prostate of intact adult male cynomolgus monkeys 3 and 14 weeks after the last subcutaneous injection of the LHRH-antagonist antide. (a) A complete atrophy of the acinar structures is evident. (b) The glandular ducts are well developed and show all signs of high secretory activity. × 360.

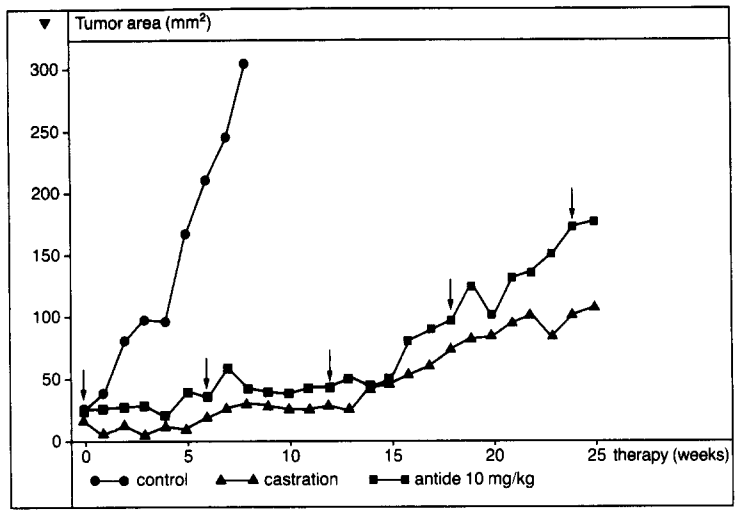

Fig. 5. The effect of the LHRH-antagonist antide on the androgen-dependent R 3327 Dunning prostate carcinoma in intact adult male rats. (↓) time of injections.

ANDROGEN-DEPENDENT R3327 RAT PROSTATIC CARCINOMA

Intact male rats were subcutaneously implanted with 2 tumors/rat and therapy was started 12 weeks after implantation. Antide was given subcutaneously every 6 weeks at a dose of 10 mg/kg. The total observation period was 26 weeks. Antide was as effective as castration in inhibiting tumor growth (Fig. 5).

CONCLUSIONS

Besides its immediate effect on testosterone production, antide has a long-term inhibitory effect on the male reproductive system in the intact rat and the intact cynomolgus monkey. In both species it was possible to induce a chemical castration, although species differences became evident in terms of the duration of such a long-term inhibitory effect.

In addition, antide was proven to be very effective in inhibiting the growth of the androgen-dependent Dunning R 3327 tumor, emphasizing the potential of this type of compound for the treatment of prostatic cancer.

Acknowledgements—The authors thank Dr S. Hasan for the analysis of LH and Mr E. Friedreich, Mrs M. Heine, Mrs B. Schilk, Mrs K. Tallér and Mrs M. Ziegler for their excellent technical assistance and Mrs Schick for typing the manuscript.

REFERENCES

1. Garnick M. B.: Leuprolide versus diethylstilboestrol for previously untreated stage D_2 prostate cancer. Result of a prospectively randomized trial. *Urology* (Suppl. 27) (1986) 21–32.
2. Pelling W. B.: Phase III studies to compare goserelin (zoladex) with orchiectomy and with diethylstilboestrol in treatment of prostatic carcinoma. *Urology* (Suppl. 33) (1989) 45–52.
3. Eisenberger M. and Abrams J.: Gonadotropin hormone-releasing hormone analogs for the treatment of prostatic cancer. *Drugs Today* **24** (1988) 241–248.
4. Crawford E. D. and Davis M. A.: Luteinizing hormone-releasing hormone analogues in the treatment of prostate cancer. In *Endocrine Therapies in Breast and Prostate Cancer* (Edited by C. K. Osborne). Kluwer, Boston (1988) pp. 25–38.
5. Akhtar F. B., Weinbauer G. F. and Nieschlag E.: Acute and chronic effects of a gondadotrophin-releasing hormone antagonist on pituitary and testicular function in monkeys. *J. Endocr.* **104** (1985) 345–354.
6. Pavlou S. N., Wakefield G. B. Island D. P., Hoffman P. G., Le Page M. E., Can R. L., Nerenberg C. A. and Kovacs W. J.: Supression of pituitary-gonodal function by a potent new luteinizing hormone-releasing hormone antagonist in normal men. *J. Clin. Endocr. Metab.* **64,** (1987) 931–936.
7. Vickery B. H.: Pharmacology of LHRH antagonists. In *Pharmacology and Clinical Uses of Inhibitors of Hormone Secretion and Action* (Edited by B. J. A. Furr and A. E. Wakeling). Bailliére Tindall, London (1987) pp. 385–408.
8. Karten M. J. and Rivier J. E.: Gonadotropin-releasing hormone analog design. Structure-function studies toward the development of agonists and antagonists: rationale and perspective. *Endocr. Rev.* 7 (1986) 44–66.
9. Schmidt F., Sundaram K., Thau R. B. and Bardin C. W. [Ac-D-Nal(2)^1, 4FD-Phe^2, D-Trp^3, D-Arg-^{6}-LHRH] a potent antagonist of LHRH, produces transient edema and behavioral changes in rats. *Contraception* **29** (1984) 283–289.
10. Phillips A., Hahn D. W., McGuire J. L., Ritchie D., Capetola R. J., Bowers C. and Folkers K.: Evaluation of the anaphylactoid activity of a new LHRH antagonist. *Life Sci.* **43** (1988) 883–888.
11. Rivier J. E., Porter J., Rivier C. L., Perrin M., Corrigan A., Hock W. A., Siraganian R. P. and Vale W. W.: New effective gonadotropin releasing hormone antagonists with minimal potency for histamine release *in vitro*. *J. Med. Chem.* **29** (1986) 1846–1851.

12. Lee Ch. H., Van Antwerp D., Heley L., Nestor J. J. Jr and Vickery B. H. Comparative studies on the hypotensive effect of LHRH antagonists in anesthetised rats. *Life Sci.* **45** (1989) 697–702.
13. Folkers K., Bowers C., Shao-bo X., Tang P. L. and Kubota M.: Increased potency of antagonists of the luteinizing hormone releasing hormone which have D-3-Pal in position 6. *Biochem. Biophys. Res. Commun.* **137,** (1986) 709–715.
14. Leal J. A., Williams R. F., Danforth D. R., Gordon K. and Hodgen G. D.: Prolonged duration of gonadotropin inhibition by a third generation GNRH antagonist. *J. Clin. Endocr. Metab.* **67** (1988) 1325–1327.
15. Puente M. and Catt K. J.: Inhibition of pituitary-gonadal function in male rats by a potent GnRH antagonist. *J. Steroid Biochem.* **25** (1986) 917–925.
16. Nestor J. J. Jr, Ho T. L., Tahilramani R., McRae G. J. and Vickery B. H.: Long acting LHRH agonists and antagonists. In *LHRH and its Analogues* (Edited by F. Labrie, A. Belanger and A. Dupont). Excerpta Medica, Amsterdam (1984) pp. 24–35.