

## SEX HORMONES AND RENAL NEOPLASIA

### *Inhibition of Tumor of Hamster Kidney by an Estrogen Antagonist, an Agent of Possible Therapeutic Value in Man*

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An estrogen antagonist of potential value in the treatment of advanced human renal adenocarcinoma, and perhaps other types of cancer in which estrogen dependency may be a factor, has been investigated experimentally. The drug, U.11,100A, a diphenyldihydronaphthalene derivative, markedly inhibited the growth of a transplanted estrogen-induced renal tumor in hamsters. The life of treated animals was substantially prolonged compared with that of control groups; after 5 weeks all animals receiving U.11,100A were alive and well with small tumors whereas all the controls injected with the solvent only had been killed because of large tumors with commencing ulceration. Seven of the 11 treated animals survived for 13 to 15 weeks before they had to be killed because of large tumors. The tumor inhibitory effect of U.11,100A was abolished largely by the simultaneous administration of estradiol, which suggests that this activity of the drug is related to its antiestrogenic properties.

**I**N RECENT YEARS ATTENTION HAS BEEN DRAWN to the possible endocrine dependency of adenocarcinoma of the kidney in man (hypernephroma) and to efforts made to treat very advanced cases of this disease with gonadal hormones, chiefly the synthetic progestational compound medroxyprogesterone acetate (Provera) and testosterone propionate.<sup>1, 2, 4, 5</sup>

Between 1959 and 1965, 38 cases with multiple advancing metastases from carcinoma of the kidney were treated with hormones at the Royal Marsden Hospital. Well-marked clinical or radiologic signs of regression were observed in eight of these patients (21%). Sev-

eral other cases showed doubtful or minor objective changes, but none of these amounted to a worthwhile clinical response. The regression rate was greater in men than in women: Six of 21 men responded, compared with only two of 17 women. In view of the rarity of spontaneous regression of renal cancer<sup>7</sup> objective signs of improvement in eight of 38 cases in the present series is considered to be due to the treatment received rather than to a spontaneous event. This is strongly supported by the shortness of the time interval between the start of hormone therapy and the first evidence of improvement.<sup>2</sup>

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#### EXPERIMENTAL BACKGROUND

In 1947 Matthews, Kirkman and Bacon<sup>20</sup> described a renal cell tumor of the kidney induced by stilbestrol in male Syrian hamsters. This work was extended by Kirkman and his colleagues in the United States<sup>13-16</sup> and also by Horning at the Chester Beatty Research Institute.<sup>8-10, 12</sup> The primary tumor was dependent upon the continued presence of administered estrogen and its development could be inhibited by treating the animal simultaneously with testosterone<sup>10, 16</sup> or progesterone.<sup>11, 16</sup> After serial transplantations at this Insti-

tute the tumor eventually grew well without exogenous estrogen.

This "independent" strain of tumor, although undifferentiated and rapidly growing, could be inhibited by cortisone treatment and totally suppressed by a combination of cortisone and Provera.<sup>4</sup> In these experiments testosterone and Provera were each without effect on the tumor but in later studies animals treated with much larger doses of Provera showed a reduction in tumor growth rate. A marked tumor inhibitory effect also was seen following adrenalectomy or orchiectomy.<sup>3</sup>

#### SEX HORMONES AND THE KIDNEY

Gonadal hormones influence normal renal structure in laboratory animals. In mice and rats castration reduces kidney weight while androgen administration leads to renal hypertrophy.<sup>17-19, 22, 23</sup> Estrogens produce degenerative changes and a fall in renal weight<sup>19, 24, 26</sup> and this action can be antagonized by progesterone.<sup>24, 25</sup>

Sex hormones also may be implicated in the induction and growth of kidney tumors. Renal cortical adenomas and adenocarcinomas are found more often in men than in women and spontaneous regression of pulmonary metastases from renal cancer occurs predominately in men.<sup>2</sup> The hormone-dependent renal tumor in the hamster is produced by estrogens, normally only in males.

The sex factor in renal neoplasia, together with the known effects of sex hormones on the normal kidney suggested that gonadal hormones might be involved in the development of spontaneous renal cell tumors and perhaps could influence the progress of renal cancer in man. This concept is now supported by the results of treating experimental and human renal cancer by endocrine methods. Since only about one in five patients with advanced renal cancer have responded to treatment with the present hormonal regime, we have continued our efforts in the experimental field to find alternative endocrine methods of treatment which may prove more successful in man.

In view of the many steroid preparations now available it is desirable to have a suitable model system with which to screen various agents of possible therapeutic value before embarking on human trials. We have continued to employ the transplantable renal carcinoma in the hamster for testing

agents of potential therapeutic value because of its response to hormone administration and to endocrine ablation procedures. As the tumor was originally induced by stilbestrol and was initially dependent upon exogenous and later endogenous estrogen for its sustained growth as a transplant,<sup>3</sup> we were interested to explore the effect on this tumor of an estrogen antagonist recently synthesized by the Upjohn Company in the United States.

#### MATERIALS AND METHODS

*Test substance:* The substance used, U.11, 100A, a diphenyldihydronaphthalene derivative, has the formula: 1 - {2 - [p - (3,4-Dihydro-6-methoxy-2-phenyl-1-naphthyl) phenoxy] ethyl} pyrrolidine hydrochloride (Fig. 1). Little work has been published so far on the compound and the pharmaceutical data given below has been provided by the Medical Division of Upjohn, Limited, Crawley, Sussex.

U.11,100A is relatively nontoxic in dogs at a dose of 1 mg/kg/day administered for 29 days. Levels of up to 100 mg/kg/day for 12 days have been tolerated by monkeys without significant toxic effect. In human volunteers doses of 60 mg/day for as long as one year<sup>6</sup> and 150 mg/day for 14 days<sup>27</sup> were not associated with serious toxic manifestations.

The compound is devoid of estrogenic, androgenic and gonadotrophin-inhibitory prop-

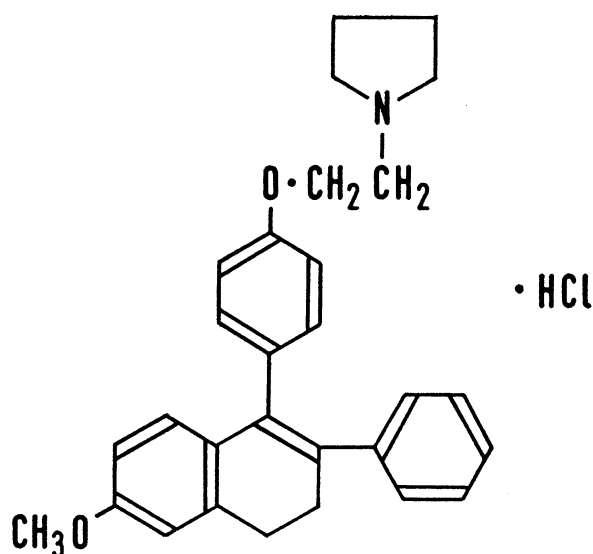


FIG. 1. Structural formula of the oestrogen antagonist, U.11,100A.

erties but has a marked antiestrogenic activity. In animal experiments, carried out in the laboratories of the Upjohn Company, it interfered with blastocyst implantation and acted as a highly effective contraceptive agent in several mammalian species. It also reduced serum sterol levels in both rats and human volunteers. The drug thus showed promise both as an oral contraceptive and as an agent of possible value in the treatment of atherosclerosis. Subsequent animal experiments, however, revealed lens changes in a proportion of dogs and rats which had received high doses of U.11,100A.<sup>6</sup>

Apart from this as yet unconfirmed report of lens changes in dogs and rats, U.11,100A appears to be free from serious toxic manifestations. If the compound were shown to be of value in the control of malignant disease, its use might be justified despite the risk of lenticular damage, especially as drug-related lens changes were not observed during nine month treatment in man.<sup>6</sup> Therefore, we have tested the drug for tumor inhibitory activity in hamsters bearing a transplanted renal tumor, induced originally by stilbestrol.

*Preliminary test of U.11,100A for antitumor activity:* The results of a pilot experiment of the same type as that described below strongly suggested that U.11,100A inhibited the growth of the estrogen-induced hamster kidney tumor. Tumor transplants in animals receiving U.11,100A for periods of from 3 to 5 weeks grew significantly more slowly than those in solvent-treated controls; rapid tumor growth was resumed about one week after treatment was suspended.

*Other chemical agents employed:* Estradiol benzoate injection BP (Oestroform, 1 mg/ml) was obtained from British Drug Houses Ltd. Arachis oil was obtained from Samooire Ltd. and dried by heating to 150°C for 30 min before use.

*Animals:* In 1947 a mutant cream-colored hamster appeared in a colony of Golden hamsters maintained at the Chester Beatty Research Institute. By back-crossing the mutant with the parent and subsequent brother-sister mating, a strain of cream-colored hamsters was obtained. This strain was employed for the present investigation because a sufficient number of Golden hamsters was not available.

Only males, 14 weeks old at the start of the experiment, were used. They were housed on wood shavings in metal cages, 10 to 12 per

cage, fed Oxoid breeding diet (supplied by Oxo, Limited) and given water ad libitum.

*Transplantable tumor of hamster kidney:* The transplantable tumor used for these experiments was one originally induced in the kidney of a male Syrian Golden hamster by the subcutaneous implantation of a stilbestrol pellet. Initially, this tumor could be transplanted successfully only to other Golden hamsters if they also were treated continuously with estrogen. Eventually, a subline of the tumor was obtained which grew successfully without administered estrogen. In the first transplant generation tumor growth was slow but in subsequent generations growth was more rapid; currently, a diameter of 1 cm is achieved in 2 to 3 weeks after implantation.

After 95 serial transplantations in the Golden hamster this exogenous estrogen-independent tumor was successfully transferred to the cream mutant strain. Initially, growth was slow but from the third serial transplantation onwards became as rapid as in the Golden hamster. For the present experiment the fifth serial passage of the tumor in cream hamsters was used.

#### DETAILS OF EXPERIMENT

Healthy tumor tissue was dissected from a cream hamster bearing a 24-day-old implant. Solid pieces measuring 1 to 2 mm in diameter were introduced by trocar and canula into the subcutaneous tissues of the right flank of 50 cream hamsters.

Only animals in which transplants were showing definite signs of growth were included in the experiment. Therefore, hamsters were examined daily and the size of tumor nodules was measured with calipers in three diameters. Hamsters were allocated to four experimental groups randomly at such time as the product of the three diameters, measured in centimeters, exceeded unity.

Between 13 and 33 days after implantation of tumor tissue, 43 of the 50 hamsters developed tumor nodules of sufficient size for inclusion in one of the four groups. Eleven of these hamsters were allocated to each of groups 1 through 3, and ten to Group 4. Three hamsters in which transplants reached the requisite size after the thirty-third day were excluded from the experiment, as were the remaining four animals in which no tumor growth was recorded by the seventh week.

In one animal of group 2 and in two of group 3 tumor growth was so rapid that the animals had to be killed within 7 days of starting treatment. These animals were excluded from the final assessment because of the exceptionally rapid growth of their tumors. To have accepted them in the control groups could only have emphasized further the tumor-inhibitory effect of U.11,100A described below.

Treatments were given to the hamsters daily, 6 days per week (Monday to Saturday, inclusive), starting as soon as tumors reached the requisite minimum size and continuing until death. U.11,100A was suspended in arachis oil and injected subcutaneously into the left flank, 1 mg in 0.2 ml arachis oil per injection. Estradiol benzoate, 0.25 mg was injected low down in the right flank at a point distant from the tumor transplant. Arachis oil, 0.2 ml, also was injected low down in the right flank.

The treatments given to hamsters in the four groups were as follows:

**Group 1**—U.11,100A in arachis oil;

**Group 2**—U.11,100A in arachis oil and estradiol benzoate;

**Group 3**—Estradiol benzoate;

**Group 4**—Arachis oil;

*Observations during experiment:* In all groups the body weight of each animal and the size of its tumor were recorded daily (Monday through Friday). Animals were observed until ulceration of the skin by a large tumor mass occurred or until they became ill, as indicated by poor general condition and loss of weight. Sick animals were killed and their tumors were dissected. Tumor and carcass were weighed separately as part of the post-mortem examination.

Mr. J. M. Mallett (Royal Marsden Hospital) examined the animals in group 1 ophthalmoscopically after they had received U.11,100A for 6 and 11 weeks, respectively. No lens changes were observed.

## RESULTS

The results of the experiment are shown in Figs. 2 through 4. More than half of the animals in groups 2 through 4 had to be killed because of large ulcerating tumors before the end of the third week and the remainder before the end of the fifth week. By contrast, all 11 animals receiving U.11,100A alone in group 1 were alive at 6 weeks. At that time

one animal in this group died but showed little evidence of tumor growth; the cause of death was uncertain. A further five animals died or were killed between the eighth and thirteenth week (Fig. 2) because of large tumors with early ulceration. The remaining five animals were killed for the same reason at the end of 15 weeks. The average survival time for group 1 was 86 days, in contrast to 15.5, 18.5 and 19 days, respectively, for groups 2 through 4.

By comparison with groups 2 through 4, the rate of tumor growth in group 1 was markedly reduced (Fig. 3). Inhibition was already evident at the end of the first week of treatment. There was considerable variation in tumor growth rate between individual hamsters but the most rapidly growing tumors in group 1 developed far more slowly than the most slowly growing tumors in group 4 (Fig. 4).

The average tumor size at death, measured as the product of 3 diameters in centimeters, was 17.3 for group 1 compared with 20.3 in group 4. This indicates that the longer survival of animals in group 1 was not attributable to their being allowed to develop tumors of greater size than those in the control group.

In this type of experiment failure of the combined weight of a hamster and its tumor transplant to increase with time is usually a reliable indication of drug toxicity. In the present study all animals in all groups gained weight at a satisfactory rate throughout the experiment; however, after the animals in group 1 had received treatment with U.11,100A for 10 weeks they became hunched and somewhat listless. Treatment was therefore suspended after the sixty-fifth injection for 9 days. During this time the animals returned to apparently normal health and vigor but 9 days after the resumption of treatment deterioration recurred. Improvement again followed withdrawal of the drug. With resumption of U.11,100A administration for the third time, deterioration was more rapid than previously and the drug was finally abandoned after only a further 3 days.

*Post mortem findings:* There was no macroscopic evidence of distant metastases in the animals of groups 2 to 4 or of drug toxicity in groups 2 and 3. None of the animals in group 1 which died or were killed up to the tenth week showed signs of metastases or drug toxicity; however, among the five hamsters which survived until the fifteenth week, four showed



survivors in the group treated with U.11,100A (group 1). The fact that metastases were seen only in this group is almost certainly due to the increased survival of treated animals, giving metastases the opportunity to develop. On the other hand, a hormonal agent (progesterone) has been reported as increasing the incidence of metastases when administered to mice bearing a transplanted mammatropic pituitary tumor.<sup>21</sup> Further experiments are being undertaken to study the possible influence of U.11,100A on the development of metastases.

Observations in rats and dogs have indi-

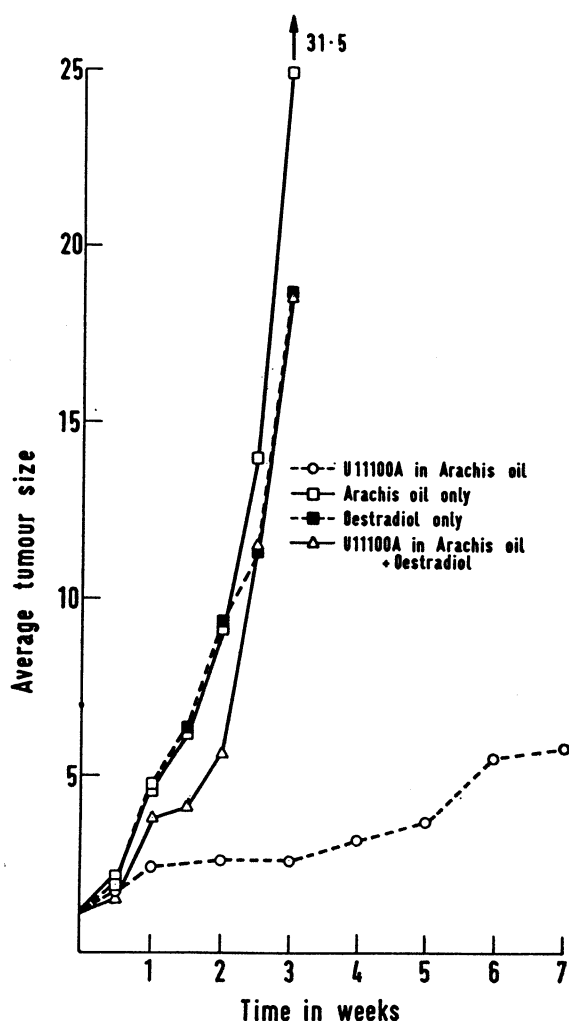


FIG. 3. Growth of tumor transplants in the four groups. Tumor size is represented as the product of 3 diameters in centimeters. Animals were allotted at random to the four groups when the product of the 3 diameters of their tumors was unity. Note the marked tumor inhibition in animals treated with U.11,100A in arachis oil.

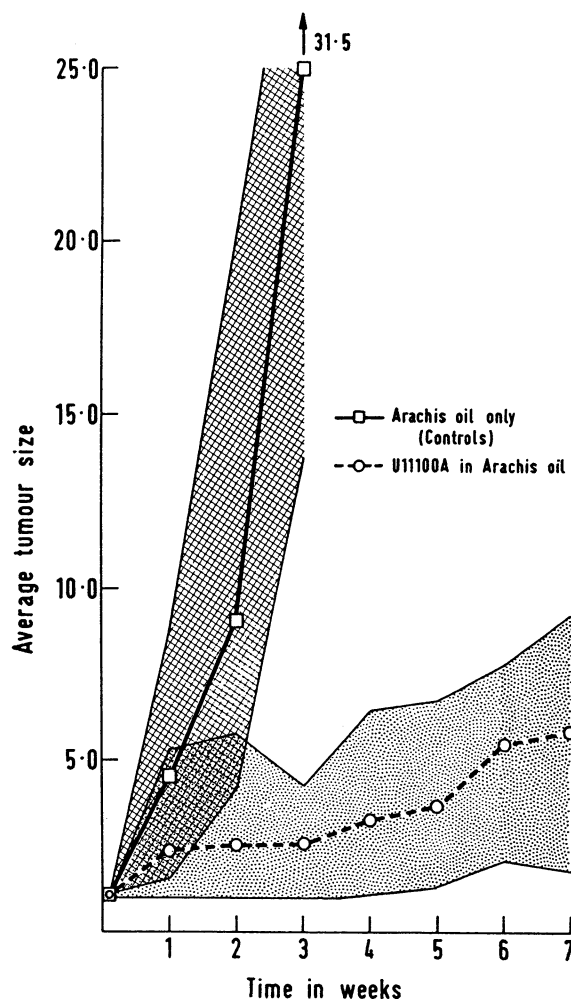


FIG. 4. Same as for Fig. 3, but showing only group 1 (receiving U.11,100A) and control group 4 (receiving solvent only). The shaded areas include all the tumor size values for individual animals.

cated that the prolonged administration of large doses of U.11,100A may be associated with cataract formation.<sup>6</sup> Hamsters receiving this drug in our experiments were examined during life, after 6 weeks and again after 11 weeks, by an ophthalmologist (J. M. Mallett); no evidence of cataract was found in any of the 11 animals composing this group. Hamsters treated with U.11,100A for longer than 10 weeks showed clinical evidence of general toxicity but the only obvious histologic changes found in postmortem tissue were congestion and hemorrhagic cyst formation in the liver and spleen in two of the five animals surviving 15 weeks.

The inhibitory action of U.11,100A against the hamster renal tumor appears to be by

estrogen antagonism since the effect was largely abolished by the simultaneous administration of 0.25 mg of estradiol benzoate daily (Fig. 3).

In view of the effect of Provera and of testosterone on patients with renal tumor metastases,<sup>2, 5</sup> the experimental results reported in this communication indicate that the

antiestrogenic property of a drug such as U.11,100A are also worth exploring in patients with advanced carcinoma of the kidney (and perhaps other forms of malignant disease, in which estrogen dependance may be a factor, e.g. carcinoma of the breast or endometrium). A clinical trial of U.11,100A has been started at the Royal Marsden Hospital.

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