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SUPPRESSION OF THE GROWTH OF THE ANDROGEN-INSENSITIVE R3327 HI RAT PROSTATIC CARCINOMA BY COMBINED ESTROGEN AND ANTIPROGESTIN TREATMENT

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Summary—The antiprogestin RU486 has been shown to inhibit the growth of a number of tumor cell lines and solid tumors which contain significant concentrations of progesterone receptor (PgR). It has been suggested that growth suppression may be due to a PgR-mediated cytotoxic effect. The R3327 HI prostatic carcinoma of the rat is considered to be a model for human prostatic carcinoma which has become resistant to androgen deprivation therapy. Since it is possible to induce high concentrations of PgR in this tumor with estrogen, it was of interest to investigate the possibility that RU486 could suppress its growth. Growth was assessed by tumor diameter, [^{3}H]thymidine uptake and histopathological appearance after 2 or 8 weeks treatment with RU486 alone, diethylstilbestrol (DES) alone, and combined RU + DES treatment as compared with control animals. Tumor growth was not affected significantly by DES treatment alone. RU486 treatment alone suppressed PgR content and resulted in only insignificant inhibition of growth. However, when significant PgR concentrations were maintained by combined treatment with DES, RU486 significantly suppressed tumor growth ($0.01 < P < 0.05$ vs controls). This was accompanied by atrophy of the glandular epithelium. The results support the hypothesis that growth suppression may be brought about by a PgR-mediated mechanism. The data suggest that it may be possible to treat androgen-insensitive prostatic carcinoma by a new form of hormonal treatment.

INTRODUCTION

The antiprogestin Mifepristone (RU486) was developed as a short-term hormonal treatment for the prevention of embryo implantation in the endometrium and for the interruption of early pregnancy [1]. It binds with high affinity to the progesterone receptor (PgR), thus competing with progestins for binding sites and preventing progestational activity. There are a number of reports of the inhibitory effects of RU486 on the growth of tumors or tumor cell lines containing significant concentrations of PgR, including the human breast cancer cell lines MCF-7 and T47D [2], mouse and rat mammary tumors *in vivo* [3, 4], and human meningioma cells *in vitro* and heterotransplanted into nude mice [5, 6]. In addition, preliminary trials of RU486 for the treatment of human breast carcinomas which have become resistant to other endocrine therapies have resulted in a modest number of responses after 3–34 weeks treatment [7, 8]. Since RU486 is ineffective in PgR negative cell lines/tumors, and inhibits the growth of hormone withdrawn cells, it has been suggested that in these lines/tumors, RU486 may inhibit growth through PgR-mediated cytotoxicity, rather than as a progestin antagonist [4, 9].

In previous work we have shown that estrogen (diethylstilbestrol, DES) treatment can induce very high concentrations of PgR in the H (androgen-sensitive) and HI (androgen-insensitive) lines of the R3327 experimental prostatic carcinoma of the rat [10]. The HI line is derived from the androgen-insensitive subpopulation of cells present in the H line tumor, and is obtained by transplanting the latter into castrated animals. It can therefore be considered as a model for human prostatic carcinoma which has become resistant to androgen deprivation

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Abbreviations: DES, diethylstilbestrol; ER, estrogen receptor; PgR, progesterone receptor; ER-EIA, ER enzymeimmunoassay; PCA, perchloric acid; RBA, radioligand binding assay; TCA, trichloroacetic acid; SN, supernatant.

therapy. Since there is a severe lack of effective treatment for androgen-insensitive prostatic carcinoma, we thought it worthwhile to examine the effects of RU486 treatment on the HI tumor. Using T47Dco cells, Horwitz [11] has shown that RU486 treatment results in the long-term suppression of cytosolic PgR replenishment. This would presumably limit the effectiveness of PgR-mediated drug activity, and we hoped that, by the induction of PgR synthesis, concurrent DES treatment of the HI tumor-bearing animals would counteract the suppression of PgR by RU486. Since the tumors are grown in castrated animals, there would be no antiandrogenic effects of DES or of RU486 in this experimental model.

In addition to the effect of long-term treatment on tumor growth *in vivo*, we examined the effects on *in vitro* tritiated thymidine uptake into DNA, on estrogen receptor (ER) and PgR concentrations and distribution between the low salt-extractable (cytosolic) and high salt-extractable (nuclear) fractions, and on tumor histopathology. As RU486 is an antiglucocorticoid as well as an antiprogestin [12], paired adrenal weight of all animals was also recorded.

MATERIALS AND METHODS

Animals, tumors and treatment

The R3327 H line tumor from which the HI tumors were derived was originally obtained from Dr N. Altman (Papanicolaou Comprehensive Cancer Center, University of Miami, Miami, FL). Copenhagen X Fischer F_1 hybrid rats were bred in our own colony from Copenhagen males obtained from Harlan Sprague–Dawley (Indianapolis, IN) and Fischer females from Charles River (St Constant, Quebec). Two groups of male offspring (24 and 36 animals, respectively) were castrated when they were 9–11 weeks old. Four days later, all animals in each group were implanted s.c. by trocar on both flanks with fragments of a single H line tumor from a donor animal castrated approx. 4 weeks previously. Animals were weighed weekly and examined to establish the time at which tumors became palpable; when they were approx. 7 mm in dia, they were measured weekly with callipers along the longest diameter and at the diameter at right angles to it. The mean diameter was used as a measure of tumor growth. Since the tumors are initially very slow-growing, the animals were placed on a restricted diet of 3–4 pellets daily 6 months after implantation, to keep their body weight below 400 g, thus limiting the deposition of fat which could affect drug distribution. The first tumors became measurable 8 months after implantation. Animals were assigned in rotation to one of 4 treatment groups when the larger tumor in each rat reached a mean diameter of approx. 1.3 cm (short-term treatment: 24 animals) or 1.0 cm (long-term treatment: 36 animals). This size was chosen to ensure sufficient tissue for analysis, while preventing the tumors from reaching a size which would result in central necrosis.

Treatment was as follows (i) DES alone: 5 μg/100 g body weight in 0.1 ml oil; (ii) RU486 alone: 2 mg/100 gm body weight in 0.1 ml oil containing 12% EtOH; (iii) DES + RU: the same doses of DES and RU486 injected at separate sites; and (iv) Control: 0.1 ml oil/100 g body weight. All injections were given s.c. 5 times weekly for 2 weeks (short-term) or 8 weeks (long-term). The final injection was given late in the afternoon of the day before killing the animals. As DES treatment results in weight loss, an *ad libitum* diet was restored to all animals during treatment.

Tumors were removed under deep ether anaesthesia using aseptic conditions, and rinsed in sterile Medium 199 without phenol red. After removal of the capsule and any areas of necrosis, a sample of each tumor was fixed for histological examination, approx. 300 mg was taken for measurement of tritiated thymidine uptake, and the remainder was frozen and stored in liquid nitrogen for receptor assay. Approx. 500 mg of thigh muscle was taken from one rat for each batch of tritiated thymidine uptake assays as negative control tissue. The adrenals from each animal were removed and weighed. Animals were killed by anaesthetic overdose.

Isotopes and chemicals

Radioactive isotopes, unlabeled steroids, scintillators and other chemicals used for PgR radioligand assays were obtained and stored as described previously [13]. Enzyme-immunoassay (EIA) kits for ER assay were purchased from Abbott Laboratories (Chicago, IL). [Methyl-^{3}H]thymidine (sp. act. 2.0 Ci/mmol) was obtained from New England Nuclear (Boston, MA) and was stored in 70% ethanol solution at -20°C for not more than 3 months. DES and unlabeled thymidine were purchased from Sigma (St Louis, MO) and

M199, without phenol red, from Gibco/BRL (Burlington, Ontario, Canada). All other chemicals were of the highest purity available. RU486 was generously provided by Roussel UCLAF (Romainville, France).

Buffers and solutions

Buffers used to prepare cytosol and nuclear extracts and for radioligand PgR assays were those described previously [13]. Additional solutions used to measure [^{3}H]thymidine uptake: 6% trichloroacetic acid (TCA); ethanol–ethyl ether (3:1) containing 1% (w/v) potassium acetate; 1.6N perchloric acid (PCA).

Receptor assays

Homogenization of tumor tissue, preparation of cytosol and nuclear extracts, radioligand binding assays (RBA) for PgR, and EIA for ER were all carried out as previously described [13]. Briefly, for PgR assays, aliquots of stripped cytosol or nuclear extract were incubated overnight at 4°C with 1–10 nM [^{3}H]promegestone (R5020). Parallel incubations were carried out in the presence of 100-fold radioinert R5020 and all incubations contained excess cortisol and dihydrotestosterone. Free and bound steroid were separated by incubating with dextran coated charcoal (DCC). The binding data were analyzed according to the method of Scatchard [14] using the program of Schwartz [15] with a Hewlett–Packard HP-97 calculator. Analysis of two curved Scatchard plots was carried out by the graphic method of Rosenthal [16].

ER-EIA assays were carried out as described in the manufacturer's instructions provided with the kit. Briefly, aliquots of stripped cytosol or nuclear extract were incubated with polystyrene beads coated with antiER monoclonal antibody. After washing, the beads were incubated with a second monoclonal antibody conjugated with horseradish peroxidase. Unbound conjugate was removed by further washing and the beads incubated with enzyme substrate solution to develop a color proportional to the amount of bound ER conjugate. Absorbance was measured at 492 nm.

Cytosol protein assays were carried out by the method of Lowry [17] and pellet DNA by the method of Dische [18].

[^{3}H]Thymidine uptake

The method was based on those of Sufrin and Coffey [19] and Carter *et al.* [20]. All procedures up to the termination of the incubation were carried out under aseptic conditions. The medium 199 used contained no phenol red.

Tumor tissue was finely minced with surgical scissors and duplicate aliquots of approx. 100 mg wet wt were suspended in 2 ml of M199 in 25 ml Erlenmeyer flasks. After equilibration at 37°C, 3 ml of M199 containing [^{3}H]thymidine was added to each flask to give a final concentration of 50 nmol (100 μCi) of [^{3}H]thymidine in 5 ml of medium. The tissue was incubated at 37°C for 5 h in a Dubnoff Incu-Shaker (Lab-Line Instruments, Melrose Park, IL) with gentle shaking (speed control 2.5), under 95% oxygen–5% carbon dioxide. Preliminary experiments had shown that [^{3}H]thymidine uptake was linear with tissue wt (25–350 mg) and time (1–5 h). After 5 h incubation [^{3}H]thymidine uptake levelled off. In all experiments parallel incubations using tissue from rat thigh muscle were carried out as a control. Incubations were terminated by the addition of 5 ml ice cold M199 containing 0.1% (w/v) radioinert thymidine and frozen at −20°C for not more than 3 days. After thawing, the tissue suspension was transferred to 15 ml corex tubes and centrifuged at 10,000 *g* for 10 min at 4°C. The supernatant (SN) was removed by aspiration and the pellet washed three times with 5 ml of 6% ice cold TCA and three times with 5 ml of ethanol–ether (3:1) containing 1% (w/v) potassium acetate. Each wash was followed by centrifugation at 10,000 *g* for 10 min at 4°C and removal of the SN by aspiration. The DNA in the pellet was extracted with 3 ml of 1.6N PCA for 20 min at 70°C and the suspension centrifuged at 10,000 *g* for 10 min. Duplicate 1 ml aliquots of the SN were used for DNA analyses by the method of Dische [18] and 50 μl aliquots were counted for radioactivity in 10 ml of PCS scintillator for 10 min or to 2% error. Results were expressed as pmol [^{3}H]thymidine/mg DNA/5 h.

Statistical analysis

The DNA yields, [^{3}H]thymidine uptake and receptor data were analyzed by the Mann–Whitney test with adjustment by the Bonferroni method for multiple comparisons. The growth curves were analyzed by Tukey's Studentized Range Test at probability levels of 5 and 1%. This test was also used to compare mean tumor size between treatment groups.

Table 1. Body and adrenal weight of animals bearing R3327 HI tumors

	Body weight (g)			
	Short-term		Long-term	
Treatment	Initial	Final	Initial	Final
Oil	357 ± 20 (6)[a]	367 ± 21	389 ± 5 (7)	418 ± 11
DES	373 ± 44 (6)	342 ± 9	378 ± 6 (6)	345 ± 18
RU486	360 ± 17 (5)	367 ± 23	379 ± 15 (6)	384 ± 10
DES + RU486	361 ± 22 (5)	342 ± 19	380 ± 13 (6)	343 ± 19
	Paired adrenal weight (mg)			
Oil	49 ± 5 (6)		58 ± 10 (7)	
DES	74 ± 8 (6)		92 ± 12 (6)	
RU486	47 ± 6 (5)		49 ± 4 (6)	
DES + RU486	90 ± 12 (5)		120 ± 19 (6)	

[a]Figures in parentheses indicate number of animals per group.

RESULTS

In order to ensure the independence of the data, the results obtained from one tumor only in each rat were used. If bilateral tumors were present, both of an appropriate size, the results from the tumor on the right side were used. This resulted in data groups of 5–7 tumors per group.

Effects of treatment on body and adrenal weight

As expected, DES treatment resulted in a loss of body weight and a gain in adrenal weight whether administered alone or with RU486 for 2 or 8 weeks (Table 1). Rats treated with RU486 alone maintained their body weight, but did not gain as much as control animals: adrenal weight was not affected by the dose given.

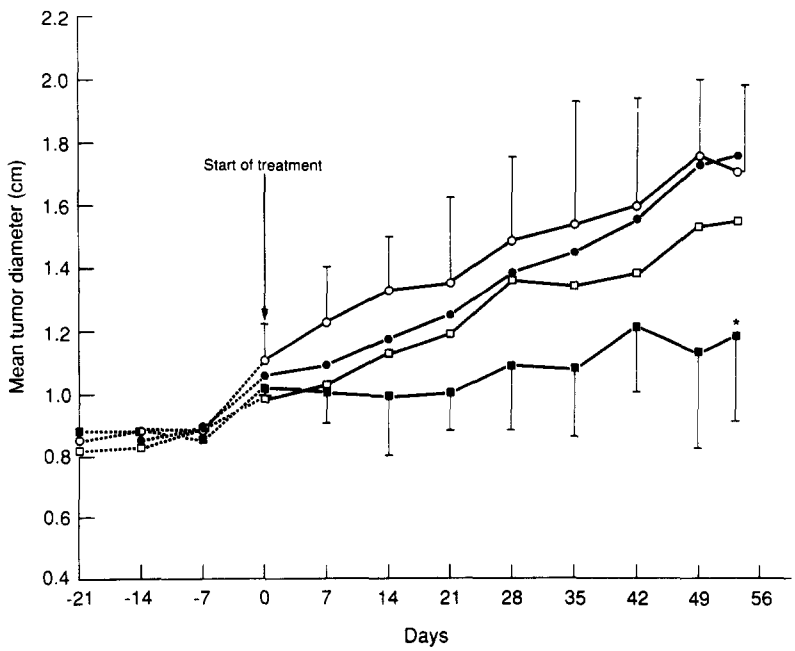

Fig. 1. Growth curves of R3327 HI tumors in control animals (○), and animals treated with DES alone (●), RU486 alone (□), or combined DES + RU486 (■). Note that not all tumors were measurable 21 days before the start of treatment. The true mean tumor diameters at −21, −14 and −7 days are therefore lower than the values shown, and the true slope of the growth curves before day 0 would therefore be steeper than that shown. Bars represent standard deviations: for the sake of clarity these have been omitted from the singly treated DES and RU486 groups. The slope of the growth curve for tumors treated with combined DES + RU486 was significantly less than that of control and DES-treated tumors ($0.01 < P < 0.05$), and at the end of the experiment the mean diameter of tumors given combined treatment was significantly smaller than that of control and DES-treated tumors (*$0.01 < P < 0.05$).

Effects of treatment on tumor growth curves

The slope of the growth curves of tumors treated with DES or RU486 alone was not significantly different from that of vehicle-treated controls, although the mean diameter of the RU486-treated tumors was lower than that of the controls at all time points (Fig. 1). Combined treatment with DES + RU486 resulted in significantly slower growth than treatment with vehicle or DES alone ($0.01 < P < 0.05$) and the mean tumor diameter

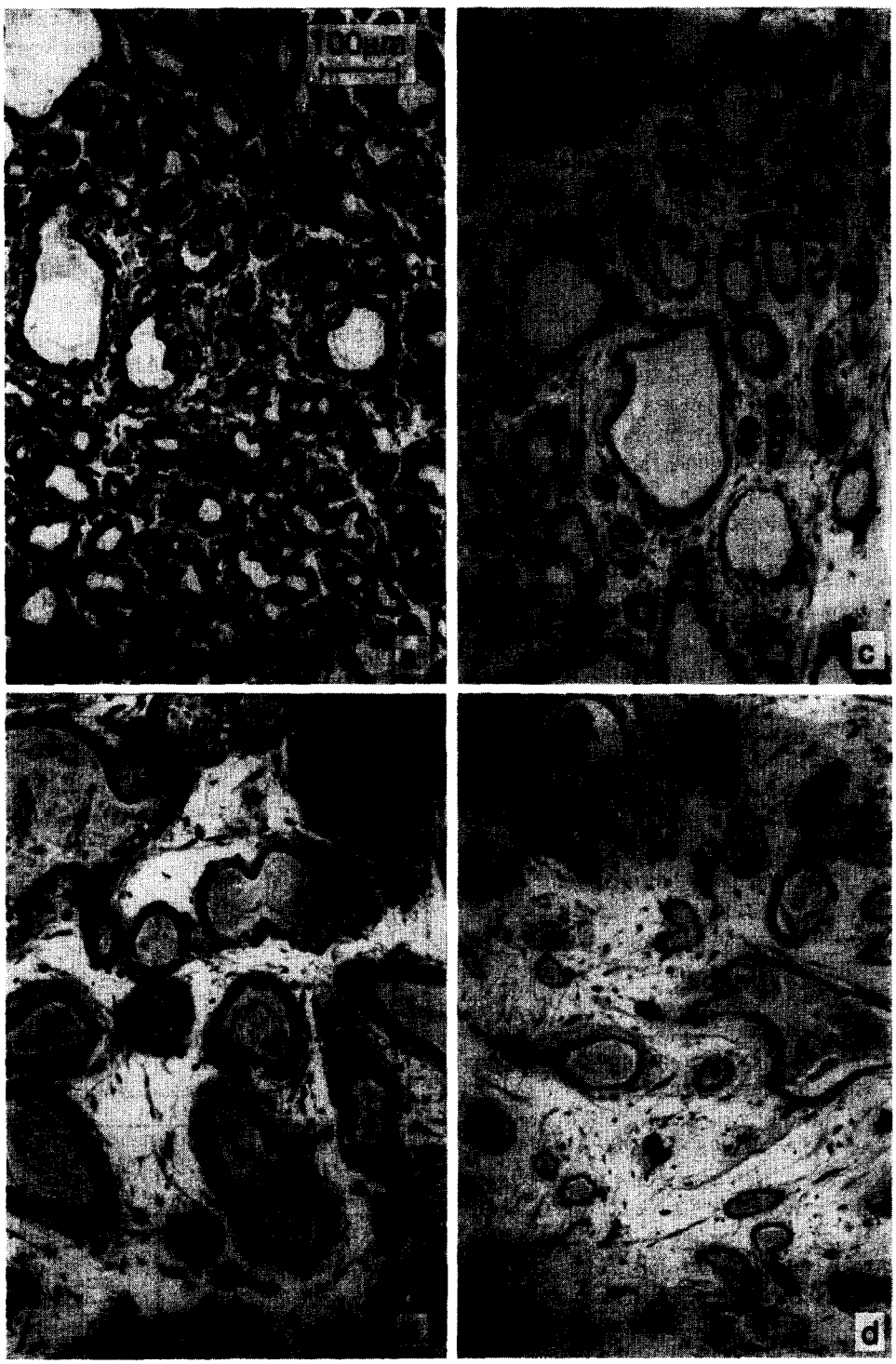

Fig. 2. Histopathology of R3327 HI tumors from control rat (a), and animals treated for 8 weeks with DES alone (b), RU486 (c) or combined DES and RU486 (d). All sections were stained with haematoxylin and eosin and were photographed at the same magnification.

at the end of the experiment was significantly lower in DES + RU486-treated tumors than in the controls or DES-treated tumors ($0.01 < P < 0.05$). Further statistical exploration of tumor size at other points suggested that slowing of growth by combined treatment occurred within 2 weeks of starting treatment ($0.01 < P < 0.05$ vs controls at 14, 21, 28 and 49 days).

Effects of treatment on histopathology

The majority of the control tumors showed typical R3327 HI histopathology i.e. they were well differentiated, with small acini of fairly regular shape and size. The acinar lumen was lined with a single layer of cuboidal epithelium. The acini were separated by fairly dense, but not extensive stroma [Fig. 2(a)]. In a few tumors, the acini tended to be more irregular in size and in one of these there was a piling up of epithelial cells in a few places. Two tumors had areas in which the stroma was more prominent, and either more dense (short-term treatment) or less dense (long-term treatment). Stromal cells had large, active-looking nuclei.

The histopathological changes due to DES treatment were more marked after long-term than after short-term treatment, and were similar to those we have reported previously [21]. The most striking effect was that on the stroma, which became more extensive and less dense. Many of the stromal cells had smaller, denser nuclei than the control tumors. The acini were usually filled with eosinophilic material, and had cuboidal or somewhat flattened epithelium, which was vacuolated in a few areas [Fig. 2(b)]. Two tumors (1 treated short-term and 1 long-term) showed multilayered epithelium in some acini.

In general, the histological appearance of the RU486-treated tumors was similar to that of the controls, although the stroma was somewhat variable in density, being less dense in some tumors than in the controls [Fig. 2(c)]. As in the controls, the acinar epithelium showed piling up in some areas of some tumors.

The histopathology of the tumors treated for 2 weeks with combined DES and RU486 was quite variable. In one, progression to poorer differentiation appeared to have occurred, as the acinar pattern was partially lost, and many areas showed multilayered epithelium. All the other tumors showed some of the same features as those treated with DES alone: the stroma was expanded and the epithelium varied from somewhat flattened to multilayered. Occasional areas of epithelial vacuolation were seen. Tumors treated for 8 weeks had a more consistent appearance. Typically, the acini were quite small with very flat epithelium, and some contained eosinophilic material. The acini were widely separated by loose stroma with scattered cells, some with round dense nuclei and others with elongated nuclei [Fig. 2(d)]. Focal vacuolation of the acinar epithelium occurred in 2 tumors. In addition to the features described, 2 tumors also contained areas of multilayered epithelium, and denser stroma.

Tritiated thymidine uptake

The uptake of tritiated thymidine by DNA was very variable, especially in the experimental groups (Table 2). The variation was most marked after short-term treatment, and no significant difference in the mean uptake was observed between groups. After long-term treatment, the mean uptake by the control tumors was almost identical to that in the short-term controls. The only significant difference in mean thymidine uptake was observed after long-term treatment with combined DES and RU486. In this group, mean uptake was significantly lower than in the controls ($149 +$ SD125 vs $483 +$ SD249 pmol/mg DNA/5 h, $P < 0.025$) and then in those treated with RU486 alone ($149 +$ SD125 vs $616 +$ SD301 pmol/mg DNA/5 h, $P < 0.025$). Uptake into muscle was consistently low: $39 \pm$ SD9 pmol/mg DNA/5 h, in the short-term treatment group ($n = 8$ assays) and $30 \pm$ SD14 pmol/mg DNA/5 h in the long-term group ($n = 11$ assays).

DNA and protein yields

None of the short-term treatments had any effect on the amount of protein or DNA per unit weight of tumor tissue (Table 3). Protein yields were also unaffected by the long-term

Table 2. *In vitro* uptake of tritiated thymidine by R3327 HI tumors

	Uptake of [^{3}H]TdR (pmol/mg DNA/5 h)	
Treatment	Short-term	Long-term
Oil	486 ± 160 (6)[a]	483 ± 249 (7)
DES	576 ± 357 (6)	339 ± 246 (6)
RU486	905 ± 648 (5)	616 ± 301 (6)
DES + RU486	737 ± 744 (5)	149 ± 125[bc] (6)

[a] Numbers in parentheses indicate number of tumors per group; [b] $P < 0.025$ vs oil; [c] $P < 0.025$ vs RU486.

Table 3. Receptor characteristics of R3327 HI tumors after short-term treatment

Treatment group (No. of tumors)	DNA yield (mg/g)	PgR Cytosol fm/mg prot.	PgR Cytosol fm/mg DNA	PgR Cytosol K_d (nM)	PgR Nuclear fm/mg DNA	PgR Nuclear K_d (nM)	PgR Total fm/mg DNA	ER Cytosol fm/mg prot.	ER Cytosol fm/mg DNA	ER Nuclear fm/mg DNA	ER Total fm/mg DNA
Oil ($n = 6$)	8.4 ± 0.7*	118 ± 32	445 ± 97	2.4 ± 1.3	31 ± 33	3.2**	476 ± 86	61 ± 22	231 ± 77	85 ± 32	315 ± 96
DES ($n = 6$)	7.3 ± 1.2	704 ± 289^a	3024 ± 1334^a	2.2 ± 1.0	312 ± 109^a	2.2 ± 1.3	3336 ± 1283^a	10 ± 6^a	42 ± 18^a	354 ± 98^a	397 ± 109
RU486 ($n = 5$)	8.3 ± 2.5	64 ± 31^c	237 ± 123	7.8 ± 3.4^a	17 ± 21	1.7**	254 ± 118^b	37 ± 27	141 ± 102	43 ± 21	184 ± 121
DES + RU486 ($n = 5$)	6.8 ± 1.9	83 ± 68^e	373 ± 277^e	6.3 ± 3.1^c	104 ± 94^{fg}	2.6 ± 0.9	477 ± 320^e	8 ± 8^{ag}	37 ± 32^{ah}	254 ± 113^{cg}	291 ± 123

*Data expressed as means ± SD; **measureable in <3 tumors.
[a] $P < 0.005$; [b] $P < 0.025$; [c] $P < 0.05$; vs oil.
[e] $P < 0.005$; [f] $P < 0.025$; vs DES.
[g] $P < 0.025$; [h] $P < 0.05$; vs RU.

Table 4. Receptor characteristics of R3327 HI tumors after long-term treatment

Treatment group (No. of tumors)	DNA yield (mg/g)	PgR Cytosol fm/mg prot.	PgR Cytosol fm/mg DNA	PgR Cytosol K_d (nM)	PgR Nuclear fm/mg DNA	PgR Nuclear K_d (nM)	PgR Total fm/mg DNA	ER Cytosol fm/mg prot.	ER Cytosol fm/mg DNA	ER Nuclear fm/mg DNA	ER Total fm/mg DNA
Oil ($n = 7$)	9.7 ± 1.7*	100 ± 46	388 ± 180	1.9 ± 1.1	37 ± 33	2.5 ± 1.4 ($n = 5$)	424 ± 197	47 ± 14	183 ± 160	83 ± 35	266 ± 88
DES ($n = 6$)	4.7 ± 0.2^a	380 ± 204^a	3264 ± 2317^a	4.9 ± 3.5	428 ± 268^a	2.1 ± 1.3	3692 ± 2396^a	4 ± 1^a	36 ± 9^a	289 ± 99^a	324 ± 100
RU486 ($n = 6$)	7.1 ± 0.8^b	19 ± 16^a	94 ± 77^a	4.7 ($n = 4$)	0	—	94 ± 77^b	30 ± 7^c	145 ± 32	82 ± 15	226 ± 37
DES + RU486 ($n = 6$)	5.4 ± 3.2^c	61 ± 24^e	372 ± 192^e	3.0 ± 1.4	15 ± 36**	1.13 ($n = 1$)	395 ± 172^f	4 ± 5^{af}	19 ± 13^{ad}	131 ± 71^d	150 ± 75^{cd}

*Data expressed as means ± SD; **detected in 1 tumor only.
[a] $P < 0.005$; [b] $P < 0.02$; [c] $P < 0.05$; vs oil.
[d] $P < 0.025$; vs DES.
[e] $P < 0.025$; [f] $P < 0.005$; vs RU.

treatments, but tumors from animals treated with DES, either alone or in combination with RU486 for 8 weeks had significantly lower mean DNA yields than control tumors (Table 4). This may have been due to the edematous effect of the estrogen resulting in fewer cells per unit wet wt of tissue. Mean DNA yields were also somewhat reduced by long-term treatment with RU486 alone.

Receptor concentrations

Both short- and long-term treatment with DES alone had the effects on ER and PgR concentrations which we have observed previously [10]. The total ER content was not changed, but there was a marked shift in ER from the cytosolic to the nuclear fraction (Tables 3 and 4). This was also observed after combined DES + RU486 treatment, but when this treatment was given long-term the shift was associated with a somewhat reduced total concentration of ER compared with all other treatments. Long-term treatment with RU486 alone reduced the cytosolic ER concentration expressed in terms of protein somewhat, but did not affect the concentration in either cell fraction as compared with controls when expressed in terms of DNA. No significant effects on ER were observed after short-term treatment with RU486 alone.

As expected, the increase in nuclear ER after DES treatment was accompanied by a marked increase in PgR concentrations as compared with controls. The mean total PgR concentration was increased 7-fold after short-term treatment and almost 9-fold after long-term treatment with DES alone. On the other hand, treatment with RU486 alone significantly reduced PgR concentrations to approximately half that in control tumors after short-term treatment and to approximately one-quarter of that in control tumors after long-term treatment. In tumors given combined RU486 + DES treatment, the DES counteracted to some extent the reduction in PgR content by RU486. In the short-term treatment group, the mean total PgR concentration was virtually identical to that in the controls, although the variation between tumors was greater in the treated group. The mean total concentration in the RU486 + DES group was almost twice that in the group treated with RU486 alone, but because of the high variation this difference did not reach significance. After long-term treatment, mean total PgR in the combined treatment group was similar to that in the controls, and significantly higher than in the group treated with RU486 alone ($P < 0.005$). Virtually all the PgR was extractable with low salt buffer i.e. was cytosolic. In addition to reducing PgR concentrations in both cytosolic and nuclear fractions, it was observed that RU486 significantly increased the K_d for binding of [^{3}H]R5020 to the cytosolic receptor. This was most marked after short-term treatment.

DISCUSSION

Although RU486 alone was ineffective in suppressing R3327 HI tumor growth, when combined with DES for 8 weeks, it showed significant inhibitory activity on [^{3}H]thymidine uptake and on increase in tumor size compared with that in control animals. This was not due to debilitation of the animals, as body weight loss was virtually identical to that in animals treated with DES alone, which did not suppress tumor growth. Animals treated with RU486 alone maintained their body weight, although they did not gain as much as the controls. Adrenal weight was not affected by the dose of RU486 administered.

As has been previously reported for DMBA-induced rat mammary tumors, RU486 treatment resulted in a fall of PgR concentration [3], possibly due to inhibition of cytosolic receptor replenishment [11]. As we had hoped, concurrent administration of DES counteracted this to some extent, restoring PgR content to control, although not to DES only-treated, levels. These effects were most marked after long-term treatment. The increased K_d for binding of the labeled ligand to PgR after RU486 treatment may reflect a change in conformation of the receptor after binding to the antiprogestin [21]. RU486 also appeared to have some effect in reducing ER levels when administered long-term with DES. Bakker *et al.* [3] observed a decrease in cytosolic ER in DMBA-induced rat mammary treated with RU486: however, in these female rats, this may have been due to increased transformation of ER to the "nuclear" form by increased circulating levels of estradiol. The mechanism for total ER reduction in the R3327 HI tumors is unclear.

The fact that significant tumor growth inhibition occurred only in those rats undergoing combined treatment (and thus with significantly higher PgR concentrations than those treated with RU486 alone) is consistent with the

hypothesis that this was a PgR-mediated effect. It is of interest that in the group treated with DES + RU486 for 8 weeks, the two tumors which showed the least inhibition of growth as indicated by their growth curves, [^{3}H]TdR tritiated thymidine uptake, and histopathology, also had the lowest concentrations of total PgR in this group. A similar effect was observed by Schneider *et al.* [4] in T61 human mammary tumor growth in nude mice. Inhibition of growth by an antiprogestin was observed only after the low concentration of PgR in the parent tumor was increased by estrogen stimulation [4]. It is also noteworthy that the maximum changes in receptor content, histopathology, [^{3}H]thymidine uptake and DNA yield in the RU and/or RU + DES-treated groups were observed only after long-term treatment, whereas increase in tumor size in the combined treatment group was significantly slowed after only 2 weeks. This suggests that growth inhibition occurred in two phases: firstly, possibly due to an excess of cell loss over cell renewal, and later by inhibition of DNA synthesis. Whether both phases were PgR-mediated is not clear.

From our previous work, we know that PgR in the HI tumor is localized in stromal cells, and is absent from glandular epithelium [22]. The effect of RU486 on the epithelium must therefore be a secondary effect mediated by the stromal cells which contain PgR. This is consistent with recent work showing that fibroblasts, or fibroblast conditioned medium, stimulates the growth of prostatic epithelial cells, presumably by the production of one or more growth factors [23].

The suppressive effect of combined RU + DES treatment on the growth of this androgen-insensitive tumor encourages us to examine the potential of this treatment for androgen-insensitive human prostatic carcinoma. On a body weight basis, the dose of DES used here (equivalent to 3.5 mg/70 kg/d) is close to the dose which has long been used as standard therapy for androgen-sensitive prostatic carcinoma. The cardiovascular side effects of DES are well known, but could possibly be minimized by giving the estrogen cyclically with continuous RU486 at a lower dose than that used here, which would be expected to produce antiglucorticoid effects in man, although other toxic side effects were not observed when a similar dose was used to treat a case of Cushing's disease [24]. This dose was higher than that used in the breast cancer trials mentioned earlier [7, 8]. Other antiprogestins are being developed which have greater antiprogestin activity and lower glucocorticoid activity than RU486 [4, 25]. One or more of these may be suitable for cancer therapy. In benign hypertrophic prostatic tissue, PgR concentrations are similar to those in the control R3327 HI tumors [26]. However, since this receptor is almost completely restricted to the stromal component [27], the concentration in highly malignant tissue is reduced [26]. Potential success of the combined antiprogestin treatment is therefore likely to be limited to a subset of patients with well differentiated tumors containing a significant stromal component. RU486 has also been shown to be mildly antiandrogenic [12], which would be an advantage in patients whose tumors contained a residual population of androgen-sensitive cells together with a predominantly androgen-insensitive population.

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