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RU 58841, A New Specific Topical Antiandrogen: A Candidate of Choice for the Treatment of Acne, Androgenetic Alopecia and Hirsutism*

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A new topically active non-steroidal antiandrogen, RU 58841 has been synthesized. It displays high affinity for the hamster prostate and flank organ (F.O.) androgen receptors. *In vivo*, when topically applied, it exerts a potent dose-dependent regression of F.O. area at a dose as low as 1 μg/animal while being devoid of antiandrogenic activity on deep accessory sex organs and of any effect on testosterone level up to 100 μg/animal. In the same species, after subcutaneous administration, it induces at the dose of 300 μg/animal, a small decrease in F.O. area equivalent to that of 1 μg applied topically and a weak systemic activity. In intact rats, no effects were observed up to 1 mg/animal whatever the route of administration. These results suggest that RU 58841 might be useful for the topical treatment of androgen-dependent skin disorders such as acne, androgenetic alopecia and hirsutism.

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INTRODUCTION

Androgens play a major role in skin disorders such as acne, hirsutism and androgenetic alopecia (male pattern baldness). Indeed, they were shown to promote sebaceous gland secretions, to induce formation of vellus from terminal hair in genetically predisposed men and to stimulate hair growth in hirsute women [1, 2]. Androgen receptors have been localized in human skin, especially in sebaceous glands and hair follicle dermal papilla [3, 4]. On the basis of these observations, some antiandrogens have been investigated to treat these disorders [5, 6]. The clinical efficacy of orally administered cyproterone acetate in the treatment of acne in females is established but the risk of feminization of foetuses arising from the systemic activity of the compound requires obligatory contraception in females of child-bearing age [7, 8]. Concerning hirsutism, there are results indicating an effect of this compound when administered orally [9]. Other antiandrogens such as spironolactone and flutamide, when given orally to females, have shown both a reduction of sebum excretion and an activity on acne and hirsutism [10]. However, the therapeutic use of these kinds of products is restricted by their possible side effects on deep androgen-dependent parameters (prostate, seminal vesicle testosterone levels and libido in the male and feminization of foetuses in pregnant females). Therefore, a topically active antiandrogen without systemic effects would be highly desirable. The compounds mentioned above have already been tested topically but the results were quite disappointing. Cyproterone acetate did not show any effect on acne and the results obtained with spironolactone were equivocal [11, 12]. The A-nor steroidal antiandrogen inocoterone acetate (RU 38882) did not display significant activity in clinical trials on acne [13]. This absence of effective topical antiandrogens led us to develop a novel non-steroidal compound, RU 58841, which displays a promising activity profile. The present paper summarizes the results obtained on its binding to the androgen receptor, its local activity and its high dissociation between local and systemic effects.

*This paper is dedicated to Dr Edouard Sakiz, in appreciation of his 40 years of commitment to research in endocrinology.

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MATERIALS AND METHODS

Synthesis: general procedure

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. Spectral data were recorded on the following spectrometers: IR, Nicolet 5 SX, in chloroform solution unless stated otherwise; NMR, Bruker AC or AM in $CDCl_3$ with TMS as an internal standard; UV, Perkin Elmer Lambda 9 in ethanol. Microanalyses were in agreement with calculated values (±0.3%) for the elements cited. Chromatrographic purifications were performed using 50–100 parts (w/w) of Merck 60 silica gel (0.04–0.063 mm). All reactions were conducted under a nitrogen atmosphere.

4-Isocyanato-2-trifluoromethyl-benzonitrile 2

A solution of aniline 1 (10 g, 53.7 mmol) in ethyl acetate (30 ml), was added slowly, at 0°C, to a 1.93 M solution of phosgene in toluene (Fluka, 33.6 ml, 64.8 mmol). The reaction vessel was set up like a distillation apparatus and was equiped to trap the HCl formed. After stirring for 30 min, the reaction mixture was allowed to return to room temperature (RT) and was then heated to reflux with distillation of part of the solvent which was replaced by fresh toluene until the temperature reached 110°C. Reflux was continued until HCl evolution subsided (total of 4.5 h). A slight precipitate was removed by filtration and the filtrate was evaporated under reduced pressure affording 11.6 g (100%) of the isocyanate 2 as an orange syrup which was used as such for the next step. IR (neat): 2268 (N = C = O) and 2233 cm^{-1} (CN).

4-(4,4-Dimethyl-5-imino-2-oxo-1-imidazolidinyl)-2-trifluoromethyl-benzonitrile 3

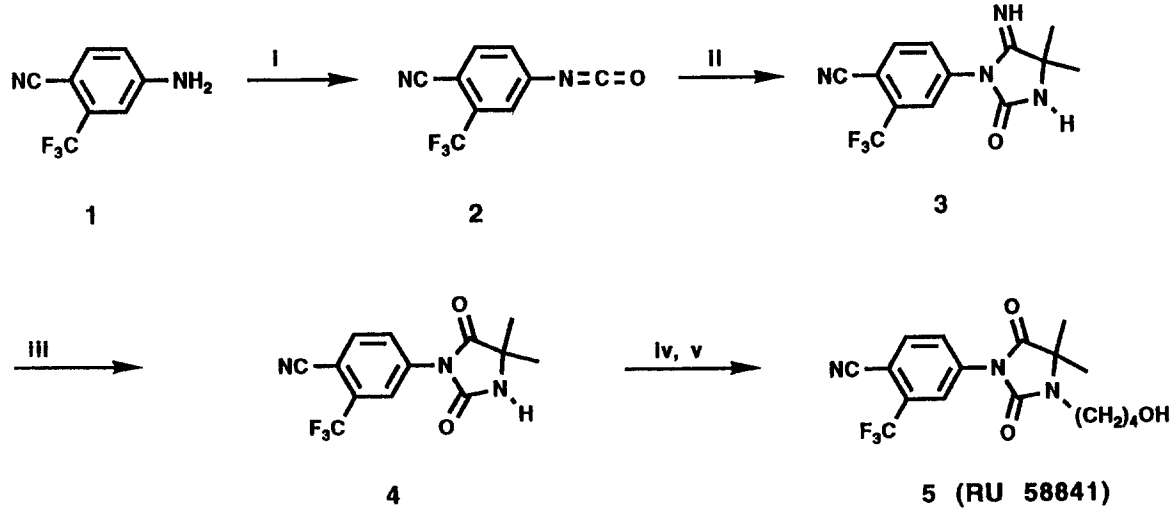
A solution of the isocyanate 2 (6.6 g, 31.3 mmol) in 1,2 dichloroethane (15 ml) was added dropwise under stirring, to a solution of 2-amino-2-cyanopropane (2.63 g, 31.6 mmol) in 1,2 dichloroethane (36 ml) and triethylamine (0.9 ml) at ice-bath temperature. After 35 min the reaction mixture was allowed to return to RT and the solvent was evaporated under reduced pressure. The residue was purified by chromatography (CH_2Cl_2–acetone, 85:15) and recrystallized from isopropanol to afford 5.378 g (58%) of 3. m.p.: 228°C; UV: λ max = 213 nm (ϵ = 17300), 272 nm (ϵ = 11600); IR (nujol): 3340, 3290 (NH), 2240 (CN), 1760 (CO), 1655 (C = N) 1606, 1570, 1502 cm^{-1} (aromatics) ^{1}H-NMR: d = 1.42 (s, 6H, gem-diMe), 8.16 (dd, 1H, H-5), 8.22 (d, 1H, H-6), 8.34 (bs, 1H, H-3), 8.44 (s) and 8.81 (s) (the NH's); analysis: C, H, F, N.

4-(4,4-Dimethyl-2,5-dioxo-1-imidazolidinyl)-2-trifluoromethyl-benzonitrile 4

The imine 3 (2.75 g, 9.3 mmol) was suspended in 6N aqueous hydrochloric acid (60 ml) and heated to reflux temperature for 35 min. The reaction mixture was cooled to RT, poured onto ice–water 1:1 (100 g) and extracted with ethyl acetate (4 × 20 ml). The organic fraction was dried over magnesium sulphate and evaporated under reduced pressure. The residue was recrystallized from isopropanol to afford 2.705 g (98%) of 4. m.p.: 210°C; UV: λ max = 256 nm (ϵ = 16200); IR: 3340 (NH), 2245 (CN), 1788, 1722 (CO), 1610, 1572, 1502 cm^{-1} (aromatics); ^{1}H-NMR: d = 1.59 (s, 6H, gem-diMe), 5.88 (s, 1H, NH), 7.95 (m, 2H, H-5 and H-6), 8.12 (m, 1H, H-3); analysis: C, H, F, N.

4-[4,4-Dimethyl-2,5-dioxo-3-(4-hydroxybutyl)-1-imidazolidinyl]-2-trifluoromethyl-benzonitrile 5

Condensation. A solution of the hydantoin 4 (2.4 g, 8 mmol) in DMF (20 ml) was added with stirring to a suspension of 50% sodium hydride in mineral oil (416 mg, 8.7 mmol) in DMF (3 ml). After hydrogen evolution had subsided, [(4-chlorobutyl) oxy]-dimethyl-*tert*-butylsilane [14] (2 g, 8.9 mmol) and sodium iodide (1.2 g, 8 mmol) were added. The reaction mixture



Scheme 1. (i) $COCl_2$, Toluene, room temperature (RT), reflux, 100% yield; (ii) 2-amino-2-cyanopropane, 1,2 dichloroethane, NEt_3, RT 58% yield; (iii) 6N HCl, reflux, 98% yield; (iv) HNa, DMF, 20°C followed by $Cl(CH)_2)_4OSi(Me)_2tBu$, NaI, 50°C overnight; (v) 2N HCl, MeOH, 40 min RT, 63% overall yield from 4.

was heated at 50°C for 18 h, poured into water (50 ml) containing potassium monophosphate (300 mg), extracted with diethyl ether (4 × 20 ml), dried over magnesium sulphate and evaporated. The residue was purified by chromatography (CH_2Cl_2–acetone, 99:1) to afford 2.8 g (72%) of the condensation product as a colourless syrup which was used as such for the next step.

Deprotection. 2N aqueous HCl (6.6 ml) was added to a solution of the preceding syrup (2.8 g) in methanol (28 ml). After standing for 20 min at RT, the reaction mixture was poured into water and extracted with chlorform. The crude product was purified by chromatography (CH_2Cl_2–acetone, 9:1) and recrystallized from isopropyl ether to afford 1.876 g of 5 (87.7%, 63% overall from 4). m.p.: 103–104°C; UV: λ max = 261 nm (ϵ = 15100); ^{1}H-NMR: d = 1.55 (s, 6H, gem-diMe), 1.67, 1.83, 3.42, 3.73 (multiplets, 8H of butyl chain), 7.91 (d), 8.01 (dd) and 8.16 (d) (aromatic protons); analysis: C, H, F, N.

Animals

Male Sprague–Dawley rats and male Swiss mice (castrated 24 h before use) were purchased from Iffa Credo (France). Male Golden Syrian hamsters, weighing 120–140 g, from Charles River (U.S.A.) were housed with a long photoperiod of 16 h light, 8 h dark.

Androgen receptor binding

Rats (weighing 180–200 g), and hamsters were castrated by scrotal route under anesthesia 24 h before removing prostate and hamster flank organs (F.O.); kidneys of mice were also removed and cytosols were prepared as described previously [15, 16]. The tritiated compounds ([1,2^{3}H]testosterone, sp. act.: 54 Ci/mmol and [5^{3}H]RU 58841, sp. act.: 24.98 Ci/mmol) were synthesized at Roussel Uclaf.

The K_a values for testosterone (T) and RU 58841 were determined after incubation of the cytosol in the presence of increasing concentrations of tritiated ligands (0.1–20 nM). Bound radioactivity was measured by the dextran-coated charcoal technique. The curves obtained were linearized by the Scatchard method [17] in order to determine the affinity constant at equilibrium (K_a) and the number of binding sites (N).

Preparation of the human androgen receptor was carried out according to a previously described method [18, 19].

Cytosol protein concentrations were determined by the Bradford technique [20].

In vivo studies

RU 58841 was administered to the animals by percutaneous (in ethanol 99.9%: France Alcool), subcutaneous (in corn oil: Mayolande, Benedicta; containing 10% of ethanol) and oral (aqueous suspension containing 0.5% methylcellulose: Colorcon) route.

Antiandrogenic activity in hamsters

RU 58841, was applied topically in ethanol to the previously shaved right F.O.; the left F.O. served as a control. Groups of 5 animals received daily, except the week-end (14 administrations over 3 weeks), RU 58841 or ethanol (control group). In order to compare the activity of the product to the effect of castration, a group of hamsters were castrated 24 h before the begining of the experiment, then they were treated topically by ethanol as above. The F.O. area was measured on days 1, 8, 15 and 19. 24 h after the last treatment, the animals were sacrificed, F.O., prostate and seminal vesicles were removed and weighed and blood was collected for seric testosterone radioimmunoassay determination (KIT TESTO-CT, CIS Biointernational).

The antiandrogenic activity of RU 58841 was also evaluated after subcutaneous treatment according to the same experimental conditions.

Antiandrogenic activity in intact rats

Rats weighing 200–250 g were treated with RU 58841 by percutaneous, subcutaneous or oral route from day 1 to 4 and from day 7 to 10. 24 h after the last treatment the animals were sacrificed, blood was collected for seric testoterone assay, prostate and seminal vesicles were removed and fixed in demineralized water containing 10% formaldehyde (Merck) for 72 h. They were then dissected and weighed.

Other organs were also removed and weighed: testes, kidneys, liver, thymus and adrenal glands.

Statistical analysis

The results were analysed using Dunnett's test [21]. Differences with $P < 0.05$ (*) and $P < 0.01$ (**) were considered significant.

RESULTS

Binding parameters of RU 58841 for the androgen receptor in different species

The binding parameters of [^{3}H]RU 58841 were compared to those of [^{3}H]T (Table 1). These two compounds gave rise, whatever the species and the target organ, to the same number of binding sites. [^{3}H]RU 58841 exhibited a high association constant (K_a) from 1.1 to 5.6 $10^9 M^{-1}$ according to species.

Table 1. Association constant at equilibrium (K_a) of [^{3}H]T and [^{3}H]RU 58841 for androgen receptor of various species

Species	K_a ($10^9 M^{-1}$) Testosterone	RU 58841
Human	1.2	1.8
Rat (prostate)	2.0	1.1
Mouse (kidney)	3.8	5.6
Hamster (prostate)	3.0	1 3
Hamster (F.O.)	0 7	1.4

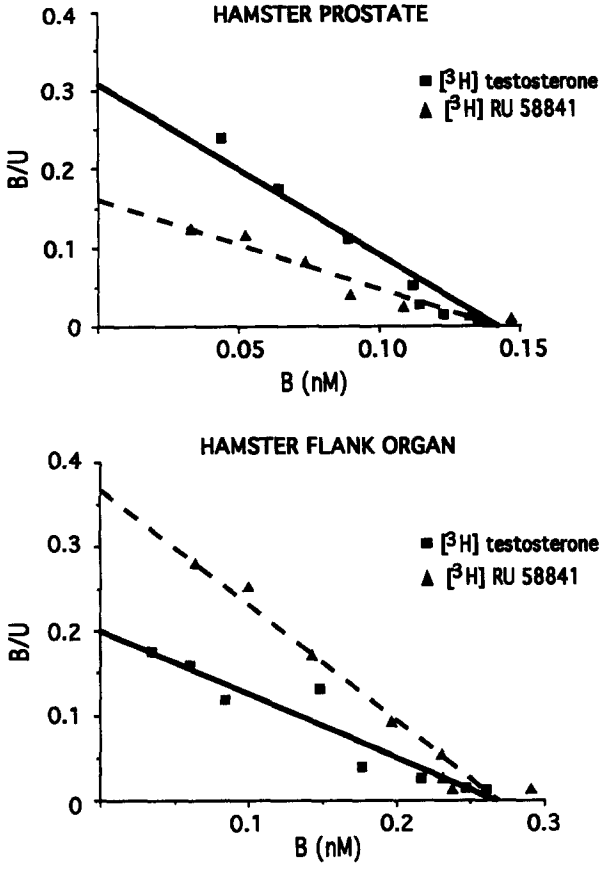


Fig. 1. Scatchard plots of [³H]T and [³H]RU 58841 binding to hamster F.O. and prostate.

It was similar or slightly higher than that of testosterone for human, mouse kidney and hamster F.O. receptors, but 2 to 3 times less in rat and hamster prostate (Fig. 1).

Antiandrogenic activity in hamsters

RU 58841, when topically applied at doses ranging from 1 to 100 μg/animal, led to a dose and time-related decrease in F.O. area [Fig. 2(a)] whereas F.O. area of the control group increased by 12%. The first significant decrease was observed at a dose of 1 μg (27.1% reduction as compared to the first day of treatment). At 100 μg a significant reduction (32.1%) already appears after 1 week and reaches 52.4%, after 3 weeks. In comparison, castration induced a rapid and large decrease in F.O. area after 7 days (51.7%), reaching a plateau value of 76.6% in 2 weeks [Fig. 2(b)]. Furthermore there was a good correlation between the decrease in area and weight of the F.O.s (Table 2). Interestingly, RU 58841 did not induce any significant variation of the contralateral F.O. and on deep androgen-dependent parameters whatever the dose.

In order to mimic a complete passage of the drug through the skin, RU 58841 was administered subcutaneously at doses of: 30, 100, 300 and 1000 μg/hamster. Only the highest doses of 300 and 1000 μg/animal led to a significant decrease in the F.O. area (27.6%). These doses also induced a decrease in prostate weight (26.9 and 34.7%, respectively), but did not affect seminal vesicle weights and seric testosterone levels whatever the dose (Table 2).

Antiandrogenic activity of RU 58841 in intact rats by various routes

RU 58841 was administered by percutaneous, subcutaneous and oral route at the doses of: 0.3, 1 and 10 mg/rat (Fig. 3). After the percutaneous treatment, only seminal vesicles were affected (41% reduction) at the highest dose of 10 mg. By the subcutaneous and oral routes, only the highest dose induced a significant

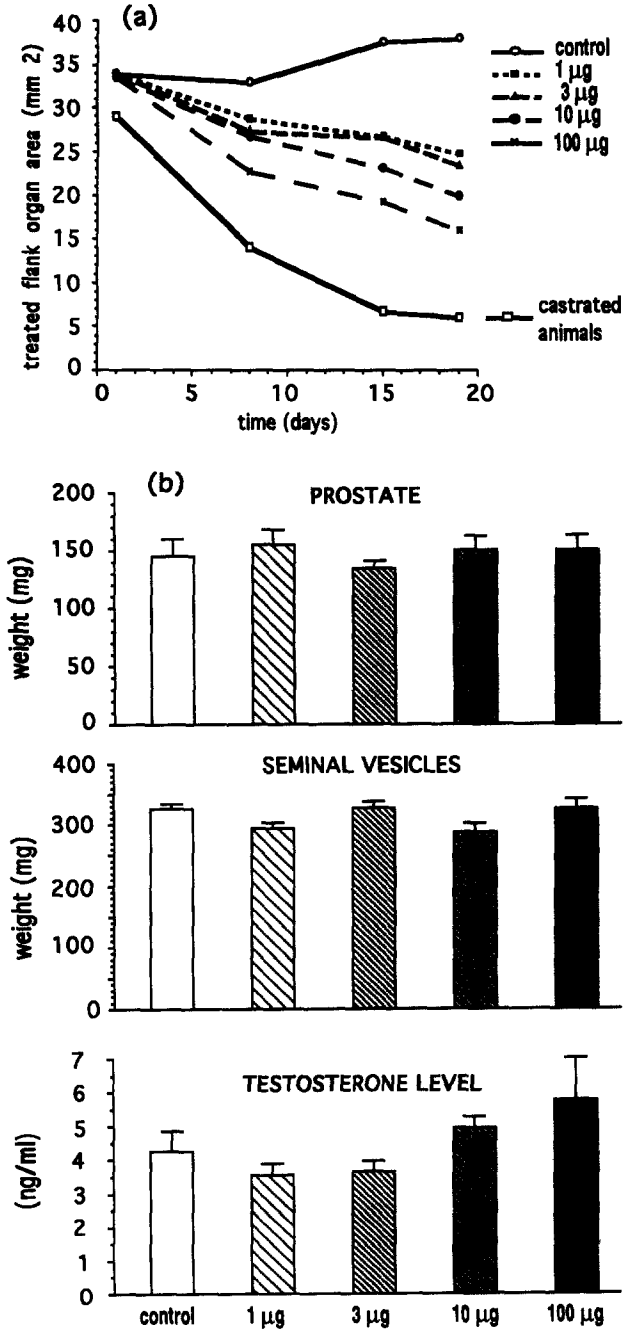


Fig. 2. Antiandrogenic activity of RU 58841 topically applied in the intact hamster. (a) (Upper panel): effect on F.O. Each point represents the mean of 5 animals. (b) (Lower panel): effect on accessory sex organs and T level. Each point represents the mean ± SEM of 5 animals.

Table 2. Antiandrogenic activity of RU 58841 locally and subcutaneously administered in the intact hamster

RU 58841 (μg/animal)	F.O. area (% of variation)		F.O. weight (% of variation)		Prostate weight (% of variation)	
	Topical	s.c	Topical	s.c.	Topical	s.c
0 (Control)	+11.8	0	0	0	0	0
0 (Castrated)	−79.3	—	−80.4	—	−69 5	—
1	−27.1	—	−39.4	—	+6.6	—
3	−32.1	—	−38.4	—	−7.4	—
10	−40 5	—	−46.6	—	+3.4	—
30	—	−7.1	—	+0.6	—	−7 2
100	−52.4	−9.4	−54.5	−12.2	+3.4	−9.7
300	—	−27 6	—	−21.4	—	−26.9
1000	—	−27.6	—	−22.8	—	−34.7

The results are expressed as % of reduction compared to the first day of treatment for the F.O. area and compared to control group for F.O. and prostate weight. Each value represents the mean of 5 animals.

decrease in prostate and seminal vesicle weights. Furthermore, the testosterone level was increased only after subcutaneous administration at the highest dose. Thus, antiandrogenic effects on deep parameters were observed only at a dose of 10 mg whatever the route of administration.

Weights of testes, kidneys, liver, thymus and adrenal glands were not modified (data not shown).

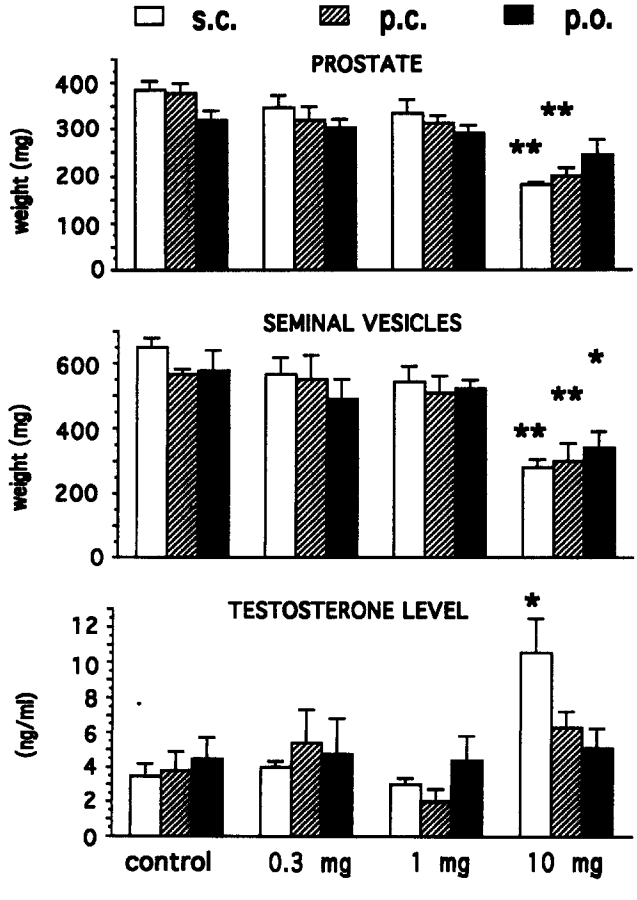


Fig. 3. Antiandrogenic activity of RU 58841 in the intact rat after subcutaneous (s.c.), percutaneous (p.c.) or oral (p.o.) treatment on accessory sex organs and T level. Each point represents the mean ± SEM of 5 animals.

DISCUSSION

RU 58841 is a new non-steroidal antiandrogen which displays a high and specific binding to the androgen receptor, equivalent or higher than that of testosterone, in different animal species as well as in humans. Its affinity is about 30 times higher than that of other non-steroidal antiandrogens such as Anandron or flutamide [22].

Its local antiandrogenic activity was evaluated on the Hamster F.O., which is widely used as a convenient screening model [23–26]. Compared to the alternate rat sebaceous gland model [27], it has the advantage of allowing the measurement of the time-dependent decrease in F.O. area on the same animal. The F.O.s, consisting mainly of androgen-dependent sebaceous tissue, are strikingly decreased after castration [28]. Contrary to several groups which have evaluated antiandrogens in castrated hamsters supplemented with testosterone propionate [23, 26], we chose to work with intact hamsters. Indeed, these physiological conditions respect the hormonal balance, and the possible influence of antiandrogens on a rise in testosterone levels can be evaluated. The local activity of RU 58841 was significant at a dose as low as 1 μg/animal and its action was limited to the site of application as shown by the absence of effect on the area of the contralateral F.O., even at the highest dose (100 μg/hamster). The local activity of some antiandrogens was evaluated by other groups on this model. Flutamide, when applied according to the conditions used in our work on one F.O. was reported by Lutsky *et al.* [23] to induce a significant effect on both F.O.s at the lowest effective dose (375 μg/day), suggesting a systemic mode of action. Other authors have used the castrated hamster supplemented with testosterone propionate in order to test the local activity of their compounds. Under these conditions, the reported effective dose for cyproterone acetate and RU 38882 was 50 μg/day [26]. Thus, RU 58841 appears to be a potent local antiandrogen compared to other compounds. Moreover, the exclusive local activity of our product was sup-

ported by the fact that no effect was observed on deep accessory sex organ weights and testosterone level up to 100 μg.

In order to confirm the high dissociation between cutaneous and systemic activity, the drug was administered subcutaneously to hamsters, to mimic a complete passage through the skin. Under these conditions, the first significant effects on prostate weight and F.O. area were seen at a dose of 300 μg/animal. Thus, the ratio between the first active systemic and local doses is between 100 and 300, giving a large safety margin to this drug.

Because a high dissociation is the main requirement for a topical antiandrogen, deep effects were also assessed in the rat. In this species the deep androgen-dependent parameters were only affected at the highest dose of 10 mg whatever the route of administration. Furthermore, when administered chronically at 15 mg/kg in castrated rats, RU 58841 did not show any adrogenic activity, as evaluated on prostate and seminal vesicle weight, whatever the route of administration (data not shown).

CONCLUSION

The exceptionally high topical activity of RU 58841 in the hamster F.O. model, combined with unprecedented selectivity with respect to systemic effects, suggest that this compound is a candidate of choice for the local treatment of androgen-dependent skin disorders such as acne, androgenetic alopecia and hirsutism. Extended preclinical evaluation is under way.

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