Effect of RU 486 on the atrophogenic and antiinflammatory effects of glucocorticoids in skin

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Received 23 August 1994; revision received 10 February 1995; accepted 10 February 1995

Abstract

Clobetasol-17-propionate (CP), a synthetic glucocorticoid (GC), reduced skin thickness in rats. Both the subcutaneous injection and topical applications of RU 486 counteracted CP-induced reduction in skin thickness. Topical application of the CP cream completely inhibited the ear edema produced by croton oil. A less potent GC, hydrocortisone-17-butyrate, also inhibited ear edema. This antiinflammatory effect was not abolished by the subcutaneous injection or topical application of RU 486. These observations suggest that GC-induced skin atrophy is mediated by glucocorticoid receptors (GRs), while the inhibition of croton oil-induced inflammation by GC is primarily related to the direct effects of GC, which are not mediated by GRs. Our findings suggest that RU 486 inhibits the atrophogenic effect of GCs without interfering with their antiinflammatory effect. Dissociation of antiinflammatory and atrophogenic activity of GC seems favorable in treating inflammatory skin diseases lacking epidermal proliferation.

Keywords: RU 486; Antiglucocorticoid; Skin atrophy; Antiinflammatory effect

1. Introduction

Glucocorticoids (GCs) are widely used in treating various skin diseases. Steroid hormones generally exert their biological effects by binding to specific receptor proteins. Glucocorticoid receptors (GRs) have been identified and characterized in the skin of various animal species [1–8]. However, there is no clear evidence that the effects of topical GCs are mediated by such receptors.

RU 486, a synthetic steroid with a high affinity for GRs, fully antagonizes the actions of GCs without exhibiting agonistic activity [9]. It has been suggested that the antagonistic activity of RU 486 is primarily the result of impaired function of the receptor-RU 486 complex [10].

The effect of GCs on skin are mostly explained by their antiinflammatory effect and their ability to inhibit DNA synthesis in the constituent cells of the skin. These two actions, one with beneficial effects in cutaneous disorders and the other sometimes with potentially adverse effects, are considered intrinsic and inseparable. Undesirable skin atrophy and secondary bacterial or fungal infections are commonly associated with topical
GCs used in the treating of inflammatory skin diseases.

In the present paper, we demonstrated that RU 486 antagonized the atrophogenic effect of CP, probably via GRs, while not affecting the anti-inflammatory effect of GCs in rat skin.

2. Materials and Methods

2.1. Chemicals

We obtained $[^3]$H dexamethasone ($[^3]$H Dex, specific activity 1.74 TBq/mmol) from Amersham International Ltd., UK. RU 486 was a gift from Roussel-Uclaf (Romainville, France). Clobetasol-17-propionate cream (0.05%, CP cream), hydrocortisone-17-butyrate cream (0.1%, HB cream) and their respective vehicles were supplied by Nippon Glaxo Ltd., Tokyo, Japan and Torii and Co., Ltd., Tokyo, Japan, respectively. The vehicles for both GC creams were emulsion ointment (oil-in-water type). RU 486 was mixed with CP cream, HB cream or the vehicles of the respective GC creams in a concentration of 0.2% (w/w). Croton oil was purchased from Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan. Other reagents used were of analytical grade.

2.2. Animals

We used 4-week-old male Wistar rats weighing 90–120 g for the skin atrophy study and 6- to 7-week-old male Wistar rats weighing 170–230 g for the croton oil-induced ear edema study.

2.3. Evaluation of skin atrophy

2.3.1. Effect of subcutaneous administration of RU 486 (Fig. 1A)

We used young rats, in which the skin is growing, so that atrophic changes in skin thickness would be clear. RU 486 in a dose of 500 μg/100 g body weight in 30% ethanol-saline (680 μl/100 g body weight) or 30% ethanol-saline only (680 μl/100 g) was subcutaneously injected to the chest area once daily for seven consecutive days. One hour after the injection of RU 486 or ethanol-saline only, CP cream or vehicle was applied to the dorsal area. The dorsal fur of experimental animals was shaved and CP cream or vehicle was

Fig. 1. Schematic diagram of the experimental designs
applied in a dose of 40 mg daily at 17:00 h for seven consecutive days to a 6 cm² (3 cm × 2 cm) hair-clipped area situated along the length of the back with the middle of the back bone as the center. Twenty-four hours after the last application, the thickness of the treated skin was measured with a dial skin thickness gauge (SM-112, Teclock, Nagano, Japan), which has an accuracy of 0.01 mm as follows: the treated skin was pinched lengthwise, and both skin surfaces were held vertically between the two plastic disks of the gauge. Then the scale was read. The skin thickness after the last topical application was compared with that just before the first injection of RU 486 or ethanol-saline.

2.3.2. Effect of topical application of RU 486 (Fig. 1B)

CP cream (40 mg), RU + CP cream (40 mg), RU cream (40 mg), vehicle (40 mg) were applied topically to the bald area of the back for seven days and then the skin thickness was measured as described above and the skin thickness after the last topical application was compared with that just before the first application.

2.4. Evaluation of ear edema

2.4.1. Effect of subcutaneous administration of RU 486 (Fig. 1C)

RU 486 in a dose of 500 μg/100 g body weight in 30% ethanol-saline (680 μl/100 g body weight) or 30% ethanol-saline only was subcutaneously injected to the chest area. One hour after the injection of RU 486 or ethanol-saline only, CP cream (20 mg) or vehicle (20 mg) was applied topically to the inside surface of one ear from the helix to the external auditory meatus. One hour after the application of CP cream or vehicle, croton oil (10%, 100 μl) in ether (v/v) was applied to the same area described above using a tuberculin syringe. Six hours after the application of croton oil, animals were anesthetized by peritoneal injection of pentobarbital. Treated and untreated ears, along with the eminence of the concha on the lower portion of the ear, were cut off and weighed. The severity of ear edema was determined by subtracting the weight of the untreated ear from that of that exposed to croton oil.

2.4.2. Effect of topical application of RU 486 (Fig. 1D)

GC cream (20 mg), RU + GC cream (20 mg) or each vehicle (20 mg) was applied to the area of ear described above. One hour after the application of GC cream, RU + GC cream or vehicle, croton oil was applied to the ear and then ears were cut off to determine the severity of ear edema as described above.

2.5. Determination of [³H] Dex binding in cytosol

The dorsal skin of rats that had been shaved of fur was excised immediately after sacrifice by cervical dislocation under anesthesia by pentobarbital. The subcutaneous tissue was removed and the portion containing the epidermis and dermis was finely minced with a scissors. Five volumes of ice-cold TEM buffer (15 mM Tris-HCl, pH 7.4, 0.5 mM EDTA, 2 mM mercaptoethanol), supplemented with 10 mM sodium molybdate and 10 mM dithiothreitol, was added to the minced tissue and the sample was homogenized five times in 5-s bursts in a polytron (Kinematika, Lucerne, Switzerland) at speed setting of 6. The homogenate was centrifuged at 105 000 g for 1 h at 4°C and the resultant supernatant was used as the cytosol.

Cytosols were incubated for 15 min at 0°C with a pellet of 1/3 volume of dextran-coated charcoal (0.25% dextran and 2.5% activated charcoal in TEM buffer: DCC) to remove free steroids. The mixture was then centrifuged at 3000 rev./min for 10 min at 4°C to remove charcoal. After 10 mM sodium molybdate and 10 mM dithiothreitol were added to the DCC-treated cytosol, an aliquot was incubated in a final volume of 0.4 ml for 16 h at 4°C with various concentrations of [³H] Dex (1–10 nM) in the presence or absence of a 1000-fold molar excess of radioinert Dex. At the end of the incubation period, 0.2 ml of DCC was added to the incubation mixture, which was allowed to stand for 10 min at 0°C. The mixture was centrifuged at 3000 rev./min for 5 min. The radioactivity in an aliquot of the supernatant was measured in a liquid scintillation spectrometer (Beckman LS-5801) with automatic standardization for correction of quenching. Specific binding was calculated by subtracting non-specific binding
(estimated in the presence of a 1000-fold molar excess of radioinert Dex) from total binding (estimated in the absence of radioinert Dex). The number of maximum binding sites (Bmax) was determined by the method of Scatchard [11]. Protein was measured by the method of Bradford, with bovine serum albumin used as the standard [12].

2.6. Determination of ligand affinities for GR

The cytosol was prepared from skin of a rat which was adrenalectomized 11 days before the experiment. An aliquot of the cytosol was incubated with 5 nM [3H] Dex and varying concentrations of unlabeled Dex, CP or RU 486 at 0°C for 16 h in a final volume of 0.4 ml. These unlabeled ligands were added in ethanol solution; final concentration of ethanol was <0.25% in all instances. After incubation, the mixture was treated with 0.2 ml of DCC and the radioactivity in an aliquot of the supernatant was counted.

2.7. Statistical analysis

Data are reported as mean ± S.E.M. They were analyzed by Wilcoxon’s rank test. A P-value of <0.05 was considered statistically significant.

3. Results

3.1. Effect of RU 486 on skin atrophy induced by GC application

The thickness of rat dorsal skin treated with vehicle of CP cream following the injection of ethanol-saline increased by 0.72 mm (Fig. 2A). Rat skin treated with CP cream following the injection of 30% ethanol-saline decreased by 0.03 mm (Fig. 2B). RU 486 injection before the application of CP cream partly prevented GC-induced reduction in skin thickness and increased skin thickness by 0.12 mm (Fig. 2C).

Topical vehicle application increased skin thickness by 0.90 mm (Fig. 3A). CP cream application reduced skin thickness by 0.06 mm (Fig. 3B). When RU 486 was added to CP cream, the atrophogenic action of CP cream was partly abolished (Fig. 3C). RU cream increased skin thickness by 0.91 mm. There was no significant difference between the effect of RU cream and its vehicle alone on skin thickness (Fig. 3D).

In preliminary experiment, histological examination of biopsy specimen revealed that the CP cream-induced decrease in skin thickness was mainly due to the atrophied dermis (data not shown).
3.2. Dynamics of $[^{3}H]$ Dex binding capacity after administration of RU 486

One hour after the injection of RU 486, $[^{3}H]$ Dex binding capacity was 10% of that observed when 30% ethanol-saline only was injected in 4-week-old rats (Fig. 4A). The binding capacity increased slightly by 6 h and was 58% of the control value after 24 h. When 6-week-old rats were injected subcutaneously with 500 µg RU 486/100 g body weight in 30% ethanol-saline, $[^{3}H]$ Dex binding capacity in skin cytosol remained low until at least 8 h after injection (Fig. 4B).

3.3. Ligand affinities for GR

The addition of CP inhibited $[^{3}H]$ Dex binding to a greater degree than the addition of Dex and the inhibitory effect of CP and RU 486 is similar (Fig. 5).
Fig. 7. Effect of topical application of RU 486 on the inhibition of croton oil-induced ear edema by CP cream. The severity of ear edema after treatment with CP cream or RU + CP cream was expressed as the percentage of the control treated with the vehicle of CP cream. Data are means ± S.E.M. (n = 5). A, topical application of vehicle of CP cream. (B) CP cream. (C) RU + CP cream. NS, no significance.

3.4. Effect of RU 486 on inhibitory effect of GC on croton oil-induced ear edema

Application of CP cream following the injection of 30% ethanol-saline only reduced the severity of ear edema induced by croton oil by 99.1% which was treated with the CP cream vehicle following the injection of 30% ethanol-saline (Fig. 6B). Application of CP cream without the injection of ethanol-saline also inhibited completely ear edema induced by croton oil (Fig. 7B). Neither injection of RU 486 before the CP cream application (Fig. 6C) nor the addition of RU 486 to CP cream (Fig. 7C) affected CP-induced inhibition of edema induced by croton oil. Topical application of the HB cream inhibited ear edema by 87%. The addition of RU 486 to HB cream did not reduce the inhibitory effect of HB cream on ear edema (Fig. 8).

4. Discussion

Topical application of GCs has been used to treat various cutaneous diseases since the report by Sultzberger and Witten in 1952 [14]. Since then, synthesis of potent GCs has markedly improved the therapy of skin diseases. However, adverse effects of GCs on skin have been reported [15–17]. Some approaches to reduce any unwanted effect of topical GC treatment have been made [18–19]. GRs have been identified in the skin and are believed to mediate a number of the effects of GCs [20–26]. However, the physiological significance of GRs in the therapeutic effects and their role in the undesirable effect of GCs on the skin remains obscure.

The skin of young rat which was treated with the vehicle of CP cream continued growing in thickness for the period of skin atrophy study (Figs. 2A, 3A). We found that CP cream had a strong atrophogenic activity in the skin of young rats. Injection of RU 486 before the application of CP cream, significantly inhibited the CP-induced decrease in skin thickness. RU 486 competed with [3H] Dex for binding sites in skin cytosol; the binding affinity of RU 486 was much higher than that of Dex but was slightly lower than that of CP. The results of competitive binding assay suggested that the effect of RU 486 on GC-induced skin atrophy was related to inhibition of binding of GC to GRs. The subcutaneous injection of 500 μg RU 486/100 g body weight did not completely inhibit [3H] Dex binding over a 24-h period. This incomplete inhibition of GC binding capacity may explain the partial inhibition of skin atrophy induced by the subcutaneous injection of RU 486. Topical application of RU 486 also inhibited the CP cream-induced skin atrophy. Topical application of RU 486 alone had no effect on skin thickness,
confirming that the inhibitory effect of RU 486 on GC-induced skin atrophy resulted from a RU-486 interference with the action of GC.

The antiinflammatory effect of GCs is believed to be related to the induction of lipocortin release but reports on GC induction of lipocortins are conflicting [27]. The antiinflammatory effects of GC are believed to be via a complicated set of biological responses. Although it was unclear which step in the process of inflammation is interrupted by GCs, the edema produced by croton oil was significantly blocked by GCs in the present study. Subcutaneous injection of RU 486 did not significantly affect the antiinflammatory potency of GCs, although the injection of RU 486 markedly reduced cytoplasmic [3H] Dex binding.

It is possible that RU 486 reduces the antiinflammatory activity of CP cream considerably, but that the residual activity is potent enough to suppress the croton oil-induced ear edema. However, this seems unlikely because RU 486 had no effect on the inhibition of ear edema induced by HB cream, which is less potent than CP. These observations suggest that the effect of GCs on croton oil-induced ear edema may not be mediated only by the GC-GR interaction, and that the direct effect of GCs, without the participation of GRs, plays an important part in their antiinflammatory effects.

In the present study, RU 486 inhibited the skin atrophy caused by GC but had no significant effect on the inhibitory effect of GC on ear edema induced by croton oil. Furthermore, our results suggest that the addition of RU 486 to GC may inhibit GC-induced skin atrophy without reducing the antiinflammatory effects of GCs. Clinically, it seems that the dissociation between antiinflammatory and atrophogenic activity of GC can confer a benefit on the therapy of obstinate skin diseases which are caused mostly by inflammation and are little accompanied with proliferation of epidermis.

Acknowledgements

We would like to thank Akie Koseki, Keiko Okada and Yuko Kadowaki for providing technical assistance. We also would like to thank Dr. D. Martini (Roussel-Uclaf) for kindly providing RU 486.

References


