Institute of Biochemistry
Biological Research Center of Hungarian Academy of Sciences
P.O. Box 521
6723 Szeged, Hungary

SÁNDOR BENYHE
MÁRIA WOLLEMANN

REFERENCES

1. W. R. Martin, C. G. Eades, J. A. Thompson, R. E. Huppler and P. E. Gilbert, *J. Pharmac. exp. Ther.* **197**, 517 (1976).
2. P. E. Gilbert and W. R. Martin, *J. Pharmac. exp. Ther.* **198**, 66 (1976).
3. J. Knoll, *Pol. J. Pharmac. Pharm.* **29**, 165 (1977).
4. E. J. Simon, J. M. Hiller, J. Groth, Y. Itzak, M. J. Holland and S. G. Beck, *Life Sci.* **31**, 1367 (1982).
5. J. Simon, M. Szücs, S. Benyhe, A. Borsodi, P. Zeman and M. Wollemann, *J. Neurochem.* **43**, 957 (1984).
6. J. Simon, S. Benyhe, K. Abutidze, A. Borsodi, M. Szücs, G. Tóth and M. Wollemann, *J. Neurochem.* **46**, 696 (1986).
7. P. D. Pezella, *Brain Res.* **273**, 297 (1983).
8. M. G. C. Gillan, H. W. Kosterlitz and J. Magnan, *Br. J. Pharmac.* **72**, 13 (1981).
9. P. L. Wood, J. W. Richard and M. Thakur, *Life Sci.* **31**, 2313 (1982).
10. S. Fürst and J. Knoll, *Pol. J. Pharmac. Pharm.* **34**, 115 (1982).

Biochemical Pharmacology, Vol. 37, No. 3, pp. 556–557, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

Reversal of the anti-inflammatory effects of dexamethasone by the glucocorticoid antagonist RU 38486

(*Received* 5 *May* 1987; *accepted* 27 *July* 1987)

The synthetic steroid RU 38486 (11β-(4-dimethyl aminophenyl) 17β-hydroxy, 17α(prop-1-ynyl) estra 4,9-dien-3-one; Mifepristone, RU 486) displays anti-glucocorticoid and antiprogesterone activities *in vivo* and *in vitro* [1, 2]. It has a high affinity for the glucocorticoid receptor *in vitro*, and is a reversible competitive antagonist, with no agonist activity.

The glucocorticoid dexamethasone has potent anti-inflammatory effects in a variety of models. For example, dexamethasone inhibits the formation of inflammatory exudate, the infiltration of leukocytes and release of inflammatory mediators (prostaglandins, leukotrienes and PAF) in the rat carrageenin pleurisy model. Since indomethacin is also active in this model some of the anti-inflammatory effects probably result from the inhibition of prostaglandin synthesis.

In this paper we report the effects of treatment with RU 38486 upon the anti-inflammatory actions of dexamethasone in this model.

Materials and methods

RU 38486 was a generous gift of Roussel-Uclaf, Romainville, France. Dexamethasone (sodium phosphate salt) was obtained from Organon Labs (Cambridge, U.K.) and carrageenin from Sigma (Poole, U.K.).

Pleurisy model

Male Wistar rats (180–220 g) were anaesthetised with ether, and 0.2 ml carrageenin suspension (1% w/v in saline) injected intercostally using a shortened, blunted 21G needle after incision of the skin. Four hours later, the animals were killed, the pleural cavities rinsed with 1 ml heparinised saline and the inflammatory exudate collected. The volume of exudate was estimated by weight, and adjusted to account for recovery of the added 1 ml by deduction of 0.85 ml, the amount recoverable following addition of 1 ml saline to non-treated rats. Leukocytes were counted, and the amount of lyso-PAF present in aliquots of cell-free exudate measured as previously described [3].

Dexamethasone, 50 μg/kg, was given subcutaneously (0.1 ml) at the same time as the carrageenin. RU 38486 was suspended in a vehicle containing 1% carboxymethylcellulose and 0.05% Tween-80, and given orally. Control animals received the vehicle alone.

Results and discussion

Dexamethasone (50 μg/kg) significantly ($P < 0.05$, Student's *t*-test) reduced the exudate volume from 1.04 ± 0.06 to 0.59 ± 0.05 g, the leukocyte infiltration from 74.5 ± 6.1 to 33.5 ± 2.5 × 10^6 cells, and the amount of lyso-PAF (the stable precursor/metabolite of PAF) present in the exudate from 13.4 ± 1.6 to 7.2 ± 1.0 ng (values are mean ± SEM, at least 15 rats, from control and dexamethasone-treated rats, respectively).

Following preliminary experiments RU 38486 was given orally 20 hr and 2 hr before carrageenin and this dosing regime resulted in a dose-dependent inhibition of the effects of dexamethasone (Fig. 1). The ID_{50} was between 20 and

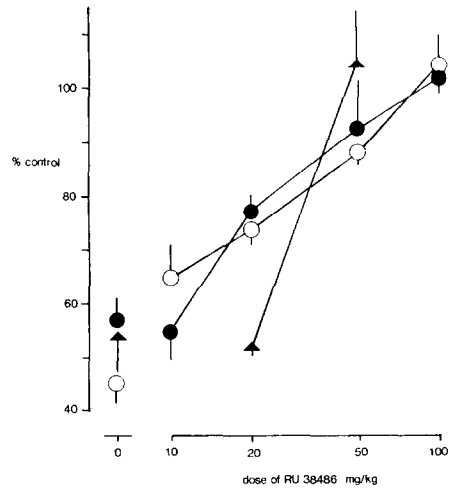

Fig. 1. Anti-glucocorticoid effect of RU 38486. Reversal of the effects of dexamethasone (50 μg/kg) upon leukocyte infiltration (○), exudate volume (●) and lyso-PAF generation (▲) by RU 38486, given as two doses of the amount shown. Each point shows the mean ± SEM from at least 5 rats for each point. The control values (100%) are as in the text, values are shown as % of this control for each parameter measured.

Table 1. Effects of dosing regime and dose of RU 38486 upon carrageenin-induced inflammation

Dose	No. doses	Cell infiltration (% control)	Exudate volume (% control)	Lyso-PAF (% control)
10 mg/kg	2	105 ± 12 (4)	95 ± 9 (4)	118 ± 33 (4)
20 mg/kg	2	132 ± 11 (8)*	122 ± 10 (8)*	103 ± 23 (4)
50 mg/kg	2	120 ± 12 (11)	125 ± 11 (11)*	119 ± 13 (10)
100 mg/kg	2	138 ± 11 (10)*	119 ± 7 (10)	143 ± 30(8)
50 mg/kg	4	134 ± 14 (9)	127 ± 9 (9)*	138 ± 9 (7)*
50 mg/kg	8	154 ± 13 (5)*	142 ± 11 (5)*	—

* $P < 0.05$.

The dose shown was given orally twice daily, the last dose 2 hr prior to carrageenin injection. Values shown are percent of values (mean ± SEM for (N) rats) obtained from vehicle-dosed rats ("controls").

30 mg/kg for each parameter, and 50 mg/kg restored the inflammation to within 10% of control (i.e. non-dexamethasone-treated) values. It is not clear why such large doses (1000-fold excess) of the antagonist were required, but it should be noted that the antagonist was given orally, whereas dexamethasone was injected subcutaneously. A dose of 10 mg/kg orally has been shown to block the effects of 10 μg/kg dexamethasone upon enzyme synthesis in the rat [1]; doses reported here are comparable. This confirms that the inflammatory actions of dexamethasone occur by means of receptor-mediated processes. The inhibitory effects of dexamethasone in carrageenin pleurisy are at least partially dependent upon its ability to inhibit lipid mediator release. This effect is probably mediated by the release of a steroid-regulated protein, lipocortin, which inhibits phospholipase A_2. RU 38486 would therefore be expected to inhibit dexamethasone-stimulated lipocortin synthesis and/or release.

Treatment with RU 38486 alone caused a small enhancement (approx. 20%) of the carrageenin-induced inflammation (Table 1). This was not markedly dose-dependent, although more prolonged treatment did increase the effect. This is probably due to inhibition of the effects of endogenous glucocorticoids, and is thus analogous to a partial adrenalectomy.

Adrenalectomised rats show a much more severe inflammatory response to carrageenin than do sham-operated animals. Exudation, leukocyte infiltration and release of lyso-PAF and eicosanoids are greatly enhanced—almost doubled—in adrenalectomised rats, but this is restored to control values by dexamethasone [3, 4]. In contrast, rats treated with RU 38486 show at most an increase of 50% of infiltration, exudation and lyso-PAF generation, and this is seen only after several days' treatment. This may reflect the dosing regime: rats were dosed only twice daily, and we have not studied the pharmacokinetics of RU 38486, although its effects are readily reversible *in vitro* [1]. In addition, it might be expected that plasma corticosterone levels would increase following treatment with a glucocorticoid antagonist, since corticosterone release is under feedback control via ACTH. Since RU 38486 is a competitive antagonist, corticosterone "breakthrough" may occur, although it should be noted that RU 38486 has a high affinity for the steroid receptor. These findings may indicate in addition that the turnover of proteins induced by steroids is quite slow, since several days' dosing is required to have a significant effect upon endogenous steroids with a dose of RU 38486 that almost completely inhibited the effects of exogenous dexamethasone. Studies using adrenalectomised animals should clarify this problem.

An additional explanation might be that the adrenalectomised, but not the RU 38486-treated animals, are lacking their adrenal medullae, site of adrenaline synthesis and release. β-adrenoceptor agonists have been shown to have actions which have potential anti-inflammatory effects, such as inhibition of increased vascular permeability [5, 6]. Glucocorticoids modulate β-receptor numbers, and inhibit agonist-induced desensitisation [7]. The implications of this require further investigation, since RU 38486-treated, but not adrenalectomised rats could potentially show β-receptor-mediated anti-inflammatory effects.

In conclusion, *in vivo* treatment with RU 38486 dose-dependently inhibits the anti-inflammatory actions of dexamethasone in the rat carrageenin pleurisy model, confirming that these actions occur by receptor-mediated processes. In addition, RU 38486 has pro-inflammatory activity, presumably due to antagonism of the effects of endogenous glucocorticoids, illustrating their important tonic role. Treatment with RU 38486 can thus produce at least a partial "chemical adrenalectomy".

Acknowledgements—We thank Dr Deraedt at Roussel-Uclaf for gifts of RU 38486. D.M. was supported by Biogen Res., Boston, U.S.A.

Pharmacology Group
University of Bath
Claverton Down
Bath BA2 7AY, U.K.

SUSAN H. PEERS*
DAVID MOON†
RODERICK J. FLOWER

REFERENCES

1. M. K. Agarwal, *FEBS Lett.* **178**, 1 (1984).
2. M. K. Agarwal, B. Hainque, N. Moustaid and G. Lazer, *FEBS Lett.* **217**, 221 (1987).
3. L. Parente and R. J. Flower, in *Inflammatory Mediators* (Eds. G. A. Higgs and T. J. Williams), p. 65. Macmillan, Basingstoke (1985).
4. R. J. Flower, L. Parente, P. Persico and J. A. Salmon, *Br. J. Pharmac.* **87**, 57 (1986).
5. E. Svensjo and G. J. Grega, *Fedn Proc.* **45**, 75 (1986).
6. E. Svensjo, C. G. A. Persson and G. Rutili, *Acta. physiol. scand.* **101**, 504 (1977).
7. A. O. Davies and R. J. Lefkowitz, *Ann. Rev. Physiol.* **46**, 119 (1984).

* To whom correspondence should be addressed.
† Present address: Department FEI, Sandoz AG, CH-4002 Basle, Switzerland.