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*Review Letter*

# Glucocorticoid antagonists

M.K. Agarwal*, B. Hainque+, N. Moustaid° and G. Lazer†

*UER Broussais Hotel-Dieu, Centre Universitaire des Cordeliers, 15 rue de l'Ecole de Medecine, 75270 Paris Cedex 06, France*

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## 1. BACKGROUND

The three principal areas of application of hormone antagonists are: clinical, diagnostic, and delineation of hormone action ([1–5] for reviews). Inhibition of synthesis of glucocorticoids in the adrenal cortex was attempted nearly three decades ago using metyrapone and aminogluthemide derivatives [3,5], and more recently with antimycotic agents [6,7]. Some 45 years ago androgens and superandrogens R 2999 and R 4841 [8–11], progesterone [12,13], oestrogens [8,14], and insulin [2] were used to oppose clinical effects of glucocorticoids, despite numerous side effects. Physiological actions of corticoids in animals could also be antagonized [15–19] but the response was equivocal [11].

It is generally admitted that adrenocorticoid hormone action proceeds via a soluble, cytoplasmic receptor in the target cell [1–5]. Thus, an 'ideal', specific, antagonist, devoid of side effects, would have to be sought by structural modification of the native hormone in relation to the receptor structure.

Correspondence address: M.K. Agarwal, 15 rue de l'Ecole de Medecine, 75270 Paris Cedex 06, France

* Directeur de Recherche, CNRS
+ Charge de Recherche, INSERM
° Doctoral student
† Recipient of INSERM International Fellowship

## 2. STRUCTURE-AFFINITY CONSIDERATIONS

Two types of structural modifications of the steroid molecule have been attempted, namely in the C and the D rings. It is clear that an alcohol function in the 11$\beta$ position is required for agonist activity. The 11$\alpha$-hydroxycortisol, the 11-keto derivative (cortisone), and the 11-deoxy analogue (cortexolone) of cortisol are antagonists in various systems in vitro [2,3,8]. Some 20 years ago, cortexolone was the only antiglucocorticoid effective on both the liver and thymus in vivo in adrenalectomised animals [2,3,8], but was transformed into an agonist after hydroxylation in the 11$\beta$ position in the adrenals of intact animals [1–3]. The 11-oxa derivatives of both cortisol and prednisolone (fig.1) cannot be hydroxylated in the 11 position and retained the antagonist activity in vivo [20]. The receptor binding correlated with biological activity in these studies.

Another school of thought lays greater emphasis on modifications in the D ring of the steroid for genesis of antagonists than on the C ring [3,5]. The 17–21 acetonides of cortisol and cortexolone are only marginally active derivatives [21] and 17–21 oxetanones of cortisol and dexamethasone are only weak antagonists of enzyme induction in hepatoma cells in vitro [20].

D ring substitution led to the synthesis of mesylates of cortisol and dexamethasone (fig.1) that form affinity labels by an irreversible

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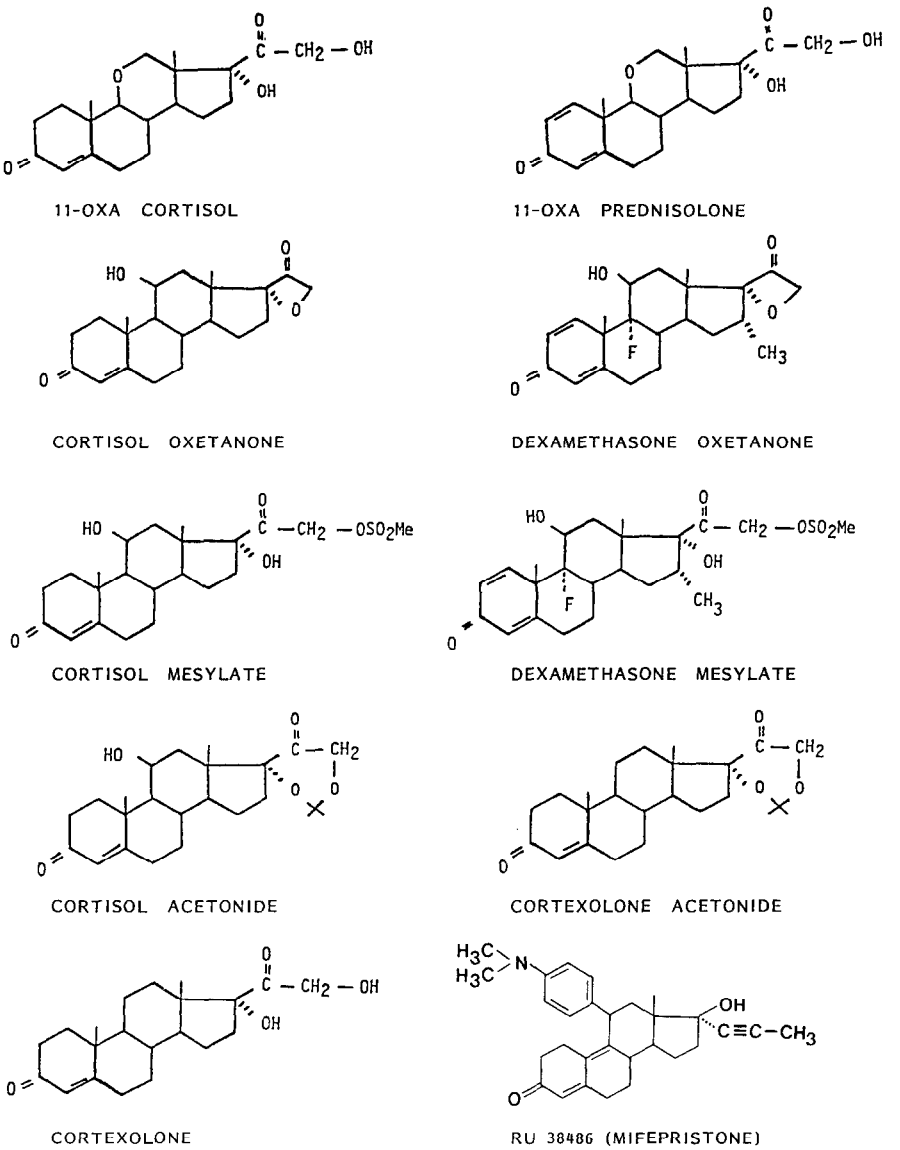


Fig.1. Structural formulae of some leading antiglucocorticoids.

blockade of the cytosolic receptor [20]. They are only partial antagonists in vitro and very toxic in vivo [20]. The 17$\beta$-carboxamides antagonised enzyme induction in hepatoma cells, but were apparently destined to oblivion since then [21].

## 3. THE EPOXIDE PATHWAY

The discovery of the epoxide pathway at Roussel laboratories [3,5] opened up a whole new area in the antiglucocorticoid field. The chemical reaction involves a modification in both the 11 position and the ketolic side chain [22,23]. A large number of derivatives has been synthesized starting from RU 26988, the ideal glucocorticoid agonist. These were subsequently used as potent probes to map the steroid-binding domain (SBD) on the receptor, and to prove the determining role of the 11 position in the agonist vs the antagonist action, although the A ring is believed to be important in binding to the receptor [3,4].

Thus, all 11$\beta$-aryl derivatives are devoid of pro-

gesterone receptor (PR) binding activity whereas the vinyl derivative has strong affinity for PR and is also a full glucocorticoid receptor (GR) agonist [4,5,8,24]. Substitution in the *para* position has a moderate effect on the SBD but *meta* substitution decreases affinity for PR leading to some dissociation between binding to the two types of receptors. Finally, the introduction of a bulky residue in the *p*-diphenyl configuration does not reduce binding to either GR or PR, suggesting that both these receptors contained a hydrophobic pocket [3,5,22,23]. Actually, it is the size of this substituent which determines the agonist vs the antagonist activity, since the synthesis of the 11$\beta$-(4-dimethylaminophenyl) derivative led to the discovery of the most potent antisteroid for both the glucocorticoid and the progesterone series [3,5,8,22,23]. What follows is essentially a catalogue of the success story of this newly synthesized derivative dubbed RU 38486, or simply RU 486, now named Mifepristone (11$\beta$-(4-dimethylaminophenyl)-17$\beta$-hydroxy-17$\alpha$-(propyl-1-ynyl)estra-4,9-dien-3-one).

## 4. A NEW RECEPTOR PROBE

In view of receptor heterogeneity [24,25], which is not due to receptor fragmentation by endogenous proteases [26–28], it was important to establish whether the agonists would saturate the same receptor populations as the antagonists. Mifepristone was bound to the same population of GR as various agonists in hepatoma cells [12], thymocytes [29–31], and rat liver [32,33].

The affinity of Mifepristone was found to be less than that of the agonist triamcinolone acetonide (TA) for rat liver GR [33] but higher than that of dexamethasone for GR in rat thymus [3,30,31] and in hepatoma cells [12,31]. The rate of dissociation of Mifepristone from the activated GR was much faster than with the agonist in thymus [29–31], as well as rat liver [33], cytosol and this was partially prevented by molybdate and phosphate buffers [29]. Paradoxically, Mifepristone-bound PR gave more stable complexes at 35°C than those obtained with progesterone in chick oviduct cytosol [34].

Whereas rat liver GR-Mifepristone complexes could be heat-activated just as well as TA-GR complexes [33], rat thymocyte GR could be activated only partially [30]. Recently, rat kidney mineralocorticoid receptor could be activated just as well in the presence of the agonist aldosterone as with the antagonist RU 26752 [35]. Thus, receptor activation is not an exclusive property of hormonal steroids, contrary to earlier studies [1–5].

Nuclear translocation of GR bound to Mifepristone was impaired in isolated rat thymus nuclei in vitro whereas agonist-GR was avidly bound to nuclei [30]. Similarly, murine thymoma Mifepristone-GR complexes exhibited diminished binding to murine mammary tumour virus DNA promoter as compared to the agonist-GR complexes [36]. These results cast doubt on the intranuclear localization of Mifepristone by autoradiography [37] where chemical analysis of the nuclear bound radioactivity was required to rule out rapid metabolic conversions in vivo [38]. Thus, intranuclear events seem more important than those in the cytosol for the physiological action of Mifepristone.

Recently, a 90 kDa protein was found to be a common component of both the GR and the PR [39] which recalls a similar hydrophobic pocket in the steroid-binding domain of the receptor for these two classes of hormones [3,5,8,24]. Contrary to the current notion, rat liver GR-Mifepristone complexes did not bind to the idiotype antibody obtained in the rabbit with TA-BSA, implying differences in the conformation and topology of the active site [40]. Chick oviduct, too, exhibited two separate binding sites, one each for progesterone and Mifepristone, contrary to the situation in calf uterus where both materials saturated an identical receptor [34]. Mifepristone did not bind to transcortin and was a better ligand than dexamethasone for chick oviduct GR [41]. All these results largely confirm some of our earliest studies on receptor multiplicity [25,26].

## 5. CELLULAR ACTION OF MIFEPRISTONE

The anabolic action (increased RNA, protein synthesis, enzyme induction, gluconeogenesis) of glucocorticoids in the liver is to be contrasted with their catabolic action (decreased macromolecular synthesis, fragmentation, cell death) in lymphatic tissues such as the thymus (reviews in [1–4]).

In adrenalectomised rats, Mifepristone abolished the induction of tryptophan pyrrolase (TP) and tyrosine transaminase (TT), as well as

gluconeogenesis, in response to synthetic glucocorticoids [3,32]. More important, the basal level of these parameters was not influenced in control animals given Mifepristone alone. Increase in TP levels, seen as a result of endogenous corticosterone secretion following ethanol administration, was also reversed by Mifepristone [42].

Although cultured hepatoma cells are much in use, neoplastic transformation permits the expression of only the TT gene [1–4]. The induction of this enzyme was reversed by Mifepristone in a dose-dependent manner in two hepatoma cell lines [43,44]. In one cell line, Mifepristone even exhibited an agonist activity [43], contrary to the liver [3,32]. No agonist activity, however, was noted when dexamethasone-mediated induction of $\gamma$-glutamyl transferase was studied in these cells [45]. Enhanced metabolism of the antagonist in liver cells, as compared to the transformed cells, may be partially responsible for this conflict [38]. In any event, these caution against reckless extrapolation in vivo of results obtained in vitro and further obviate the validity of hepatoma cells as a viable model for normal liver functions.

The catabolic effect of glucocorticoids (inhibition of uridine incorporation) was reversed by Mifepristone in thymocytes in vitro [3,32] at a time when the formation of dexamethasone-receptor complexes in the cytoplasm and the translocation of this complex into the nuclei were also impaired by the antagonist [32]. Mifepristone also reversed dexamethasone-mediated inhibition of growth in a human cervix cell line [46], of the synthesis of procollagen mRNA in chick skin fibroblasts [47], and of the induction of Epstein-Barr virus in Daudi lymphoma cells [48].

The inhibitory effect of dexamethasone on the humoral as well as the cellular immune response was overcome by Mifepristone [49]. Similarly, this material reversed hydrocortisone-mediated inhibition of prostacyclin synthesis in rat aorta [50]. It also overcame the inhibition of prostacyclin and thromboxane $A_2$ after the administration of progesterone which is known to be a glucocorticoid antagonist [1–5], possibly due to its antiprogestin activity [50,51].

In the pituitary from the neonatal rat which, contrary to the adult animal, totally lacks the mineralocorticoid receptor, aldosterone exhibits corticotropic activity and inhibits ACTH release which is reversed by Mifepristone [52]. On the other hand, glucocorticoids can induce hypertension in the rat and this can also be antagonised by Mifepristone which exhibits no intrinsic activity of its own [53]. Finally, hydrocortisone was shown to increase angiotensinogen synthesis in Reuber hepatoma cells in vitro and this too could be prevented by Mifepristone [54]. These data suggest that glucocorticoids may be involved in the regulation of arterial tension in the mammal although so far this has been supposed to be controlled mostly by the brain mineralocorticoid receptor [55]. In fact, oral administration of Mifepristone abolished vasoconstriction produced by topical application of glucocorticoids in normal men [56]. In addition, it also lowered the intraocular pressure in the rabbit eye [57].

Clinically, Mifepristone administration reversed the atrophy of the levator ani muscle in the rat seen in response to endogenous or exogenous glucocorticoids [58,59]. Mifepristone also reduced symptoms of Cushing's syndrome due to ectopic ACTH secretion [60,61].

Administration of Mifepristone at midnight in the human inhibits the dexamethasone blockade of the pituitary adrenal axis [62,63]. When given alone at 10 a.m., it increased cortisol levels at 4 p.m. which returned to normal by 8 a.m. the following morning [64], but had no effect if administered at 4 p.m. [63]. Increased ACTH and cortisol levels were also observed in the monkey [65,66]. Mifepristone-mediated disinhibition of the dexamethasone-blocked retrocontrol was also evident in pituitary cells in vitro where it exhibited no intrinsic activity [67]. Thus, Mifepristone may be used as a diagnostic tool for the pituitary-adrenal axis in various situations [64,68].

## 6. FUTURE TRENDS

It is evident from the foregoing that the epoxide pathway, leading to the synthesis of Mifepristone has provided an entirely new vision in the antiglucocorticoid field. It has no inherent agonist or antagonist activity, no known toxicity or side effects, and high metabolic degradation albeit of low availability in vivo. Thus, it comes close to being an ideal glucocorticoid antagonist, despite associated antiprogestin activity [51]. For the first time, then, an antiglucocorticoid, active in vivo,

has finally been found. It is currently being used as a starting point for the synthesis of more specific antiglucocorticoids and of steroids with inverse C-13 configuration [69,70]. The in vivo significance of receptor heterogeneity [24,71,72], of endogenous proteases [26–28], and of phosphorylation [5,73], can all be fruitfully scrutinized with Mifepristone. Since it can sensitize mice to endotoxin lethality for prolonged periods of time [71], Mifepristone appears to possess effects that do not necessarily involve the glucocorticoid receptor and this should be kept in mind in future studies.

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