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Antihormones in Health and Disease

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Antihormones in Health and Disease

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Preface

Molecular endocrinology is a rapidly advancing field. Since hormones modulate cell function by binding to specific receptors in the membrane and/or the intracellular compartment, recent efforts have centered on the synthesis of molecules that specifically and selectively bind to their appropriate carriers. The synthesis of analogs endowed with antagonist activity has been important not only to understand the molecular action of the hormone but, perhaps even more important, a number of these materials have been used clinically to control hormone-dependent syndromes. The present volume brings under one cover recent advances in a number of areas and provides a larger perspective for future effort within a conceptual framework.

Mineralocorticoid antagonists have permitted the purification of the endogenous receptor for the first time and one such molecule has even replaced the natural hormone, aldosterone, as the 'ideal' ligand for delineation of the receptor structure and function. Earlier synthesis of RU 38486 had revealed remarkable similarity in the structure of receptors for the glucocorticoids and progestins. Much progress has now been made to dissociate the antiprogestin action of RU 38486 from its antiglucocorticoid activity. Since RU 38486 is used clinically both for contraception and the treatment of Cushing's disease, the dissociated antihormones are obviously of paramount importance more so because they can be of much use in breast cancer, glaucoma, immunodeficiency, obesity, wound healing, hypertension, etc. Recently, RU 38486 has been shown to aggravate shock, and to oppose sexual behavior, opening up even more areas of potential application.

The synthesis of new antiestrogens and antigonadotropins may herald a new era in the control of breast cancer, fertility control, contraception, to mention only a few. The demonstration that epitestosterone, the natural

metabolite of the male sex steroid in the human, antagonizes androgen action may have far-reaching implications for the organism. Modulation of androgen action by diet opens up an entirely novel analysis of aging and senescence. Similarly, the use of antibodies to study the receptor structure, and contraception, are contemporary themes of much potential.

Conceptually, hormone antagonists have challenged the classical notion of receptor-mediated hormone action. Thus, antiglucocorticoids and antiestrogens may oppose the action of the natural hormone in a manner quite independent from a mere blockade of processes triggered by the native agonists. Similarly, posttranslational modifications of the receptor may well explain physiological diversity despite a primary structure whose similarity spans different organs and species. Clinically, a number of hormone-dependent dysfunctions, as well as contraception and fertility control, have already undergone some revolution in recent years with the aid of newly synthesized antihormones. Since the next decade is bound to yield ever more potent products, the time is ripe to cope with the ethical, psychological, and legal implications associated with medical progress for the well-being of the individual at all stages of life.

Paris, September 1990 *M.K. Agarwal*

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From RU 38486 towards Dissociated Antiglucocorticoid and Antiprogesterone

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Introduction

A major breakthrough in the search for progesterone and glucocorticoid antagonists was the discovery that some 11β substituted 19-norsteroids, particularly the 11β aryls [1, 2], display both potent antiglucocorticoid and antiprogesterone activities in vivo and, in some cases, also a moderate antiandrogenic property [3, 4]. The most famous and extensively studied among them, RU 486 or RU 38486 [11β-(4-dimethylamino phenyl)-17β-hydroxy-17α-(1-propynyl)-4,9-estradien-3-one], has recently been commercialized in France as an antiprogestin (Mifegyne) in first-trimester pregnancy interruption [5, 6]. However, this type of compound possesses other potential therapeutical applications. In fact, as a result of its antiprogesterone component, it should be suitable for the treatment of hormono-dependent tumors [7–10] and through its antiglucocorticoid component it could also be used for the treatment of Cushing's syndrome [11]. A broader therapeutical indication would involve all the diseases in which glucocorticoids might be more or less involved such as glaucoma, obesity, immunodeficiency, wound healing, and hypertension [12–17]. However, the presence of multi-antihormonal activities in the same molecule may limit its clinical use especially for long-term therapy. That is why the search for dissociated antagonists would be desirable [18, 19]. To this end about 300 molecules have been synthesized. The structural modifications involved concern essentially positions 11β, 17

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and 10β of the steroid skeleton. These molecules have been studied systematically using a three-step screening: (a) evaluation of their relative binding affinity (RBA) for the 5 classes of steroid receptors; (b) antagonistic or (and) agonistic activities on cellular models; (c) determination of the antihormonal profile in vivo on rapid acute tests. The most interesting compounds resulting from this screening were investigated more extensively.

The aim of this paper is to describe the different structure-affinity and structure-activity relationships which led to the discovery of RU 43044, a pure antiglucocorticoid, and of the dissociated antiprogestins RU 46556 and RU 49295. Furthermore, a complete biochemical and pharmacological profile of these 3 compounds compared to that of RU 38486 will be presented.

Results

Table 1 defines the models which were used in order to establish a complete biochemical and pharmacological profile for a given test compound. In the first instance, compounds were tested systematically on the following screening: In vitro determination of the relative binding affinity (RBA) for the steroidal receptors [20] and evaluation of the antiglucocorticoid activity on rat thymocytes [3, 21]. Compounds were tested in vivo for their antiprogestin and antiglucocorticoid activities, by evaluation of the abortive potential [4, 22] and inhibition of dexamethasone-induced hepatic tryptophan pyrrolase activity [3, 23] in the rat. On these latter models, the compounds were tested with single oral doses of 3 and 10 mg/kg, respectively, doses at which RU 38486 is totally active.

Products which displayed an antagonistic activity on one of these in vivo tests, comparable to RU 38486, were then submitted to the other models listed in table 1. Sprague-Dawley rats and New Zealand rabbits were used. In all experiments the test compounds were administered orally suspended in aqueous solution containing 0.25% carboxymethylcellulose and 0.2% polysorbate 80. Dexamethasone (Dexa) by the i.p. or oral route was administered using the same vehicle. Progesterone and testosterone propionate were injected by the s.c. route in sesame oil containing 5% benzyl alcohol.

Structure-Affinity Studies

As shown in tables 2 and 3, most of the test compounds bind to GR, PR and AR receptors. The RBAs for ER and MR were negligible and are not

Table 1. Routine models used for the evaluation of hormonal and antihormonal activities: RBA for the steroid receptors[1] and human orosomucoid[2]

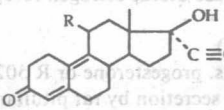
Rabbit uterus progesterone, rat thymus glucocorticoid, rat prostate androgen, rat kidney mineralocorticoid and mouse uterus estrogen receptors (respectively, PR, GR, AR, MR and ER)	
Plasma human orosomucoid (HO)	
Antiprogesterone activities vs. progesterone or R 5020	
In vitro	LH secretion by rat pituitary cells
In vivo	abortion and deciduoma formation in the rat
	endometrial transformation in the rabbit
Antiglucocorticoid activities vs. dexamethasone	
In vitro	uridine incorporation in rat thymocytes
	ACTH secretion by rat pituitary cells
in vivo	hepatic tryptophan pyrrolase (TP), thymus and cotton granuloma weights in the rat
Antiandrogen activities vs. testosterone	
In vivo	prostate weight in the rat

[1] The RBAs of the test compounds were evaluated as described previously [20]. The RBAs of progesterone, dexamethasone, testosterone, aldosterone and estradiol for PR, GR, AR, MR and ER, respectively, were taken arbitrarily equal to 100. Incubation time for PR, AR, MR and GR was 24 h at 0 °C and 5 h at 25 °C for ER. Bound radioactivity was measured by the dextran-coated charcoal (DCC) technique.

[2] Human plasma diluted (1/100) in 0.01 *M* Tris-HCl (pH = 7.4) 0.25 *M* sucrose buffer (TS) was incubated for 4 h at 0 °C with 20 n*M* of tritiated RU 38486 in the presence of concentrations of cold RU 38486 or test compounds. Bound radioactivity was measured by the DCC technique. The RBA of RU 38486 was taken equal to 100.

shown. Some of the compounds which were found to have interesting in vivo activities were tested for their RBA for the human α_1-acid-glycoprotein (orosomucoid). Indeed, we discovered that this protein, whose serum concentration is of the order of 1 g/l, binds RU 38486 at 0 °C with an association constant at equilibrium of $10^7\ M^{-1}$. This type of binding has been sought in the plasma of various animal species, but as shown in figure 1, only human plasma displayed a strong and saturable binding for RU 38486 [24]. It has also been shown that this binding to orosomucoid, considerably affects the pharmacokinetic parameters of RU 38486 in humans, as compared with those in rats and monkeys. So, the apparent initial volume of distribution (AIVD) and the clearance determined in humans are 20 and 100 times lower, respectively, than in the precited species [25, 26]. In the absence of a predictive animal model to determine the way in which this binding might affect the antihormonal activity of our products, we are in the process of

Table 2. Influence of 11β substitution on RBAs for steroid receptors and human orosomucoid

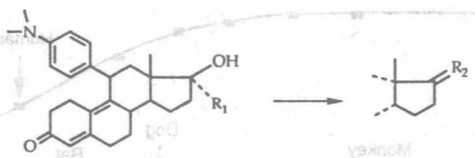


RU Code	R	Relative binding affinity % PR	GR	AR	HO
39 628	H	3	0.2	0.3	-
38 275	Me	240	60	0.3	-
38 502	t-Butyl	0.7	50	0.2	-
39 115	C_6H_5	65	240	11	-
38 604	p-Me-C_6H_4	295	295	10	-
38 955	p-OMe-C_6H_4	510	300	19	-
38 810	m-OMe-C_6H_4	15	250	0.4	-
39 171	p-SMe-C_6H_4	450	170	12	85
40 221	m-SMe-C_6H_4	13	160	6	-
38 486	p-NMe_2-C_6H_4	530	300	23	100
40 555	p-MeNiPr-C_6H_4	240	160	5	0
49 292	p-MeCO-C_6H_4	370	200	30	6
39 329	p-C_6H_5-C_6H_4	230	110	0.6	3
44 253	p-Me-C≡C-C_6H_4	100	58		-
43 780	p(-C_6H_5-C≡C)-C_6H_4	11	5	<0.1	-

comparing, in humans, the antiglucocorticoid activity (ACTH stimulation) of RU 40555 (table 2) with that of RU 38486. The biochemical and pharmacological profile of RU 40555 is very similar to that of RU 38486, except for the fact that the former compound does not bind to orosomucoid. The results of the experiment could be determining for the selection of future antihormones.

Table 2 summarizes the effects of variations in the 11β-substitution pattern on RBAs for PR, GR and AR. This type of study had already been

Table 3. Influence of D ring substitution on RBAs for steroid receptors and human orosomucoid



RU Code	R_1	Relative binding affinity % PR	GR	AR	HO
40 222	H	20	30	60	~160
51 566	CH_3	130	90	30	-
38 473	C≡CH	350	235	70	315
38 486	C≡C–CH_3	530	300	23	100
40 070	C_6H_5	165	180	4	0
39 746	C≡C–C_6H_5	250	90	0.4	0
48 541		55	6	4	-
40 016		4	120	7	-
50 502		410	40	9	440
45 781		310	50	13	120
46 299		220	95	4	-
45 149		280	22	16	210

performed [27, 28] and had shown that both the GR and the PR possess a large hydrophobic pocket able to accommodate bulky 11β-substituents. Now, whereas the depth of this pocket is similar for the two receptors, as deduced from the RBAs of RU 39329 and RU 43780, the cross-section in the vicinity of the steroid seems to be quite different, as inferred from the RBAs of RU 38502. In particular, we have already shown that the hydrophobic pocket present in the glucocorticoid receptor partially overlaps with the 10-position of the steroid, suggesting that the 10β-methyl group of

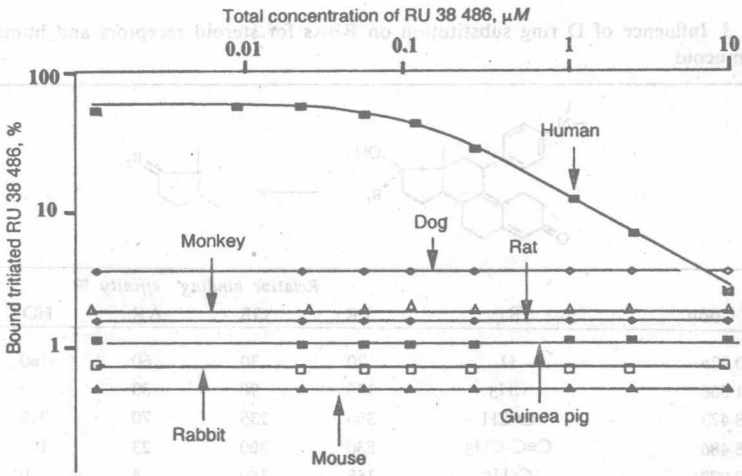


Fig. 1. Binding of RU 38 486 to plasma of various species. Plasma diluted in TS buffer (1/100) was incubated for 4 h at 0 °C with 20 n*M* of ^{3}H-RU 38 486 in presence of increasing concentrations of cold RU 38 486. Bound radioactivity was measured by the DCC technique.

classical corticoids protrudes into the same hydrophobic pocket [27]. This reasoning has incited us subsequently (see below) to substitute the 10β position in order to achieve differential binding in favor of the GR. Still looking at table 2, one can see that for substituted phenyl rings to the 11β position, the transfer of a substituent from the para to the meta position (compare RU 38955 vs. RU 38810 and RU 39171 vs. RU 40221) leads to a drop of the RBA for the PR without influencing too much the high binding for the GR. Unfortunately, RU 38810 and RU 40221 were found to be, respectively, 3 and 10 times less active than RU 38486 as antiglucocorticoids in vivo. At this point one can make the following comment: In order to be of any use for mapping purposes, a substituent must have a well-defined location in the space surrounding the steroid nucleus. In other terms, it should have as few degrees of freedom as possible. For the substituents listed in table 2, the first 3 (H, Me and t-butyl) obviously fulfill this criterion, as a consequence of their axial symmetry. Phenyl and para-substituted phenyl also fulfill it, because of the restricted rotation of the phenyl ring. Indeed, it has been shown by energy calculations, as well as by X-ray cristallography [28], that the phenyl ring closely eclipses the C_9-C_{11} bond. The meta-substi-

tuted phenyl group, however, will be of little use for mapping, because the meta-substituent can be either on the front side, overlooking the steroid framework, in what one could call an 'endo' position, or on the 'back side', away from the steroid, in an 'exo' position. So far, X-ray diffraction studies have not been performed on these kinds of compounds, and it is therefore not known if one of these two positions is occupied preferentially or if it is just random.

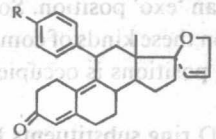
The effect of various D ring substituents has been explored in terms of receptor and HO binding. As shown in table 3, the introduction in the 17α position of substituents with increasing length, up to the propynyl (RU 38486), induces a gradual increase in the binding to both PR and GR, whereas more bulky or longer substitutions (RU 40070 and RU 39746) only slightly affect the affinity for these 2 receptors. However, 17α substitution profoundly modifies the binding to AR and HO (RU 38473 compared to RU 39746). In vivo, as expected according to their receptor interactions, these compounds display a pharmacological profile similar to that of RU 38486, some of them being as active as the reference compound.

Other structural modifications carried out on the D ring (table 3) have proved to be more interesting. In fact, the inversion of the C17 configuration of RU 38473 leads to RU 40016 which still exhibits a strong RBA for GR but a poor RBA for PR. Unfortunately this product is at least 3 times less antiglucocorticoid in vivo than RU 38486. Nevertheless, this type of structural modification could constitute a lead for discovering new dissociated antiglucocorticoids. In contrast, the introduction of various spirocyclic groups gives rise to molecules which retain a potent affinity for PR while displaying reduced binding to GR. Furthermore, these derivatives generally exhibit a strong affinity for HO as exemplified by RU 50502. In vivo, these steroids, except for RU 45149 show an abortive activity in the rat similar to that of RU38486.

In order to optimize this antiprogesterone activity, RU 46299 was chosen as a reference compound and the dimethylamino group on the 11β-phenyl was replaced by various other substituents as shown in table 4. Most of the resulting compounds displayed an improved dissociation in favor of the PR. RU 49295 and RU 46556 have been found to be more potent abortifacients than RU 38486 and were selected for extended investigation [22].

Before ending this section devoted to structure-affinity relationship we have studied the predictive value of our animal receptor screening by evaluating the RBA of RU 38486 for the progesterone receptor of several

Table 4. RBAs for steroid receptors and human orosomucoid of a series of 11β substituted-19-norsteroids with a 17-unsaturated spiroether group



RU Code	R	*Relative binding affinity %* PR	GR	AR	MR	HO
38 486		530	300	23	0.5	100
46 299	Me_2N	220	95	4	0.5	-
49 095	MeO	270	25	9	0.5	11
46 556	MeS	240	40	2.5	0.4	29
49 295	MeCO	520	25	43	4.3	3
49 723	EtS	100	25	2.4	2.2	4

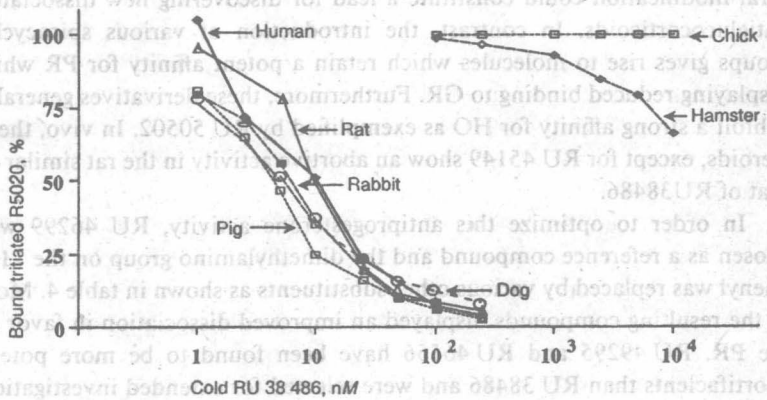


Fig. 2. Binding of RU 38486 to progesterone receptor in several species. Uterine cytosols (rat, rabbit, dog, pig and hamster), oviduct cytosol (chick) and human breast cancer cell cytosol (T47D) were incubated with ^{3}H-R 5020 (a potent progestin) in presence of increasing concentrations of cold RU 38486. Bound radioactivity was measured by the DCC technique.

species including humans. As shown in figure 2, it can be observed, as previously described, that RU 38486 is not recognized by PR of chick oviduct [29, 30] and hamster uterus [31, 32]. This observation is confirmed by the fact that RU 38486, at oral doses up to 100 mg/kg, is devoid of any antiprogesterone activities on abortion and deciduoma formation tests in the hamster (results not shown). Furthermore, preliminary results indicate that this lack of binding to PR is a general feature of all the 11β-substituted steroids tested so far, whereas the GRs of these 2 species both bind this series of compounds including RU 38486. Therefore, these results raise the general problem of the predictive value of animal receptor screening for selecting drugs suitable for use in human health. Although the steroid binding domain of the rabbit PR used in our screening presents an almost perfect homology with the human PR [33] it is felt necessary, in order to improve our screening, to select the compounds on human receptors. This goal is now at hand, insofar as all the human steroid receptors have been cloned and expressed within the last 5 years [34–37].

Dissociated Antiprogestins

As mentioned above, RU 46556 and RU 49295 have proved so far to be the most interesting derivatives. Their antiprogesterone and antiglucocorticoid activities have been evaluated in vitro on different cellular models as well as in vivo on acute and chronic tests and compared to those of RU 38486. Their antihormonal activities in vitro are reported in table 5. On thymocytes, RU 46556 and RU 49295 are, respectively, about 10 and 20 times less antiglucocorticoid than RU 38486 in reversing the inhibitory effect of Dexa on uridine incorporation. On pituitary cells the antiprogesterone activity of RU 46556 on R 5020-induced LH release is about 4 times more potent than that of RU 38486 and RU 49295. On these cells, RU 46556 and RU 49295 are, respectively, at least 3 and 30 times less antiglucocorticoid than RU 38486 on Dexa-inhibited ACTH release. Consequently, these 2 compounds are considerably more dissociated antiprogestins than RU 38486, as deduced from the IC_{50} ratios. In fact, RU 46556 and RU 49295 are about 13 and 30 times more dissociated than RU 38486. This dissociation, as shown in table 6, is confirmed in vivo in acute (abortion, TP) as well as on chronic (deciduoma formation, thymolysis) tests. Indeed, RU 46556 and RU 49295, administered orally, are more active than RU 38486 as antiprogestins whatever the test, while being, respectively, at least 50 and 100 times more dissociated as deduced from the ED_{50} ratios of the chronic tests. One can note that at the highest dose used

Table 5. Antiprogesterone (LH) and antiglucocorticoid (ACTH, thymocytes) activities of RU 38486, RU 46556 and RU 49295 on rat cells

Compounds	Thymocytes uridine incorporation IC_{50} n*M*	Pituitary cells, IC_{50} n*M*		$\frac{IC_{50}\ ACTH}{IC_{50}\ LH}$
		LH	ACTH	
RU 38486	40	8	30	3.7
RU 46556	500	2	100	50
RU 49295	900	8	>1,000	>125

Antiprogesterone activity (LH release): Pituitary cells were prepared and incubated as described previously [22]. 10 n*M* of R 5020 were incubated in presence of the test compounds. The concentrations which inhibited by 50% (IC_{50}) the potentiating effect of R 5020 on LH release were determined. Antiglucocorticoid activities: (a) ACTH release: pituitary cells were incubated with 10 n*M* of Dexa in the presence of the test compounds. The ability of these compounds to reverse the inhibitory effect of Dexa on ACTH release was evaluated (IC_{50}). (b) Uridine incorporation: thymocytes were incubated with 50 n*M* of Dexa in the presence of the test compounds. The ability of these compounds to reverse the inhibitory effect of Dexa on uridine incorporation into RNA was evaluated (IC_{50}).

(100 mg/kg) these compounds are totally devoid of any antiglucocorticoid activity on Dexa-induced thymus involution. The antiprogesterone activity of these compounds has also been evaluated in the rabbit by measuring their inhibitory effect on the progesterone-induced endometrial transformation [4, 38]. As shown in table 7, RU 38486 displays an antihormonal activity while being devoid of any agonistic activity. Surprisingly, the two other derivatives, when administered alone at a dose of 30 mg/kg, induce a slight but significant endometrial proliferation scored 1.4–1.7 U according to the McPhail scale. This effect was well reproducible with RU 49295 but was observed only once out of 3 experiments with RU 46556. This seemingly agonistic activity might be explained by the fact that testosterone also causes a small endometrial transformation of 1 McPhail unit and that these two compounds, unlike RU 38486, display a significant androgenic activity on the rat prostate weight from a dose of 10 mg/kg (fig. 3). This hypothesis is under investigation using a potent and specific antiandrogen.