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A Novel Approach to the Discovery of Non-systemic Anti-inflammatory Steroids; Antedrug

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Therapeutic use of anti-inflammatory steroids is limited due primarily to their systemic suppressive effects on pituitary function and the immune system. To overcome the clinical limitation, a new approach toward the discovery of non-systemic anti-inflammatory steroids is based upon the antedrug concept introduced by this laboratory. The new concept describes locally active agents which are designed to undergo a predictable biotransformation to inactive metabolites upon entry into systemic circulation from the applied site. Thus, true antedrugs are devoid of systemic adverse effects. In a continuing effort, 16α-carboxylate and isoxazoline derivatives of prednisolone have been synthesized and screened. In the croton oil-induced ear edema bioassay, the following relative potencies were obtained setting hydrocortisone=1.0; **3a**, 1.5; **3b**, 3.1; **4a**, 4.0; **4b**, 12.2; **5b**, 8.2; **6b**, 11.2; **7a**, 1.9; **7b**, 4.1; **8a**, 3.3; **8b**, 6.8; **9a**, 0.7; **9b**, 8.6; **10a**, 2.6; **10b**, 7.4. Results of the five-day bioassay indicated that, in contrast to the parent compound, the novel steroidal antedrugs did not significantly alter body weight gain, thymus weights, adrenal weights or plasma corticosterone levels. Taken together, the antedrug concept appears to be a fundamentally sound strategy for the separation of local anti-inflammatory activity from systemic adverse effects.

Key word : Antedrug, Prednisolone derivative, Croton oil ear edema, Receptor binding

INTRODUCTION

Since the discovery by Hench and coworkers in 1948 that cortisone could be used to abrogate most of the debilitating symptoms of rheumatoid arthritis, glucocorticoids have been used in therapy for a wide spectrum of inflammatory diseases (Hench *et al.*, 1950). The list includes the rheumatic diseases, allergic diseases, plus a variety of ophthalmic and dermatological disorders (Green and Lutsky, 1986; Avery and Woolfrey, 1997).

Although glucocorticoids have attained mainstay status in the therapy of many inflammatory and allergic conditions, their use especially on a chronic basis is restricted by a myriad of deleterious systemic effects that they precipitate. Among these systemic effects, increased susceptibility to infection, suppression of the hypothalamic-pituitary-adrenal axis, osteoporosis, hypertension, diabetes and growth retardation in children have proven to be quite debilitating. Therefore, the early task of steroid chemists was directed to synthesize corticosteroids which were more potent anti-inflammatory agents but had reduced incidence of side effects. As a result of this research effort, very potent synthetic corticosteroids with virtually no salt-retaining activity have been produced. However, the development of powerful corticosteroids in the 1950s and 1960s have not been entirely successful, since the increase in anti-inflammatory potency of the synthetic corticosteroids was accompanied by proportional increase in undesirable systemic toxicities (Lee *et al.*, 1989). These shortcomings are largely inherent in the nature of corticosteroids themselves; not only do corticosteroids possess multiple biological activities, but the structural requirements for various glucocorticoid activities appear to be overlapping and inseparable (Lee and Soliman, 1982; Popper and Watnick, 1974).

This situation calls for new approaches for the development of anti-inflammatory steroids with significantly improved local to systemic adverse activity ratios, for primarily local and/or topical applications. Two important new approaches have been dominant: (i) a rational approach to improve the lipophilicity of potent corticosteroids for local use, by masking the hydroxyl group, (ii) synthesis of non-systemic anti-inflammatory steroids by introducing metabolically labile functional group at strategic positions on the steroid nucleus in accordance with the antedrug concept. This review gives a brief account on the evolution of anti-inflammatory steroids and the recent progress of non-systemic steroids designed under the antedrug concept.

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As a result of the early research effort to synthesize high potency anti-inflammatory agents, very potent semi-synthetic corticosteroids with minimal salt-retaining activity have been introduced. Table I depicts the structure of hydrocortisone with arrows indicating the four favorite positions at which medicinal chemists successfully introduced a double bond, methyl group or halogen atom on the steroid nucleus. The resulting high potency anti-inflammatory steroids which are used as pharmaceutical products are presented with their relative glucocorticoid and mineralocorticoid activities.

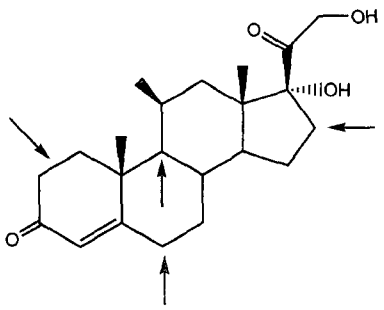
There were historically successful structural modifications of hydrocortisone that enhance glucocorticoid potency. One of the earliest modifications was the introduction of fluorine at the 9α-position to increase the binding affinity to corticosteroid receptors and to retard the oxidation of the proximal 11-hydroxy group. It resulted in agents such as fluorocortisone (1954). The introduction of a double bond between carbon atoms 1 and 2, as in prednisolone (1955), led to increased potency with reduced salt-retaining activity. This structural feature has endured in most subsequent corticosteroid drugs. The incorporation of a C-6 methyl, to prevent hydroxylation at the position as in methyl prednisolone (1956), led to the synthesis of more potent agents with longer duration of action. The introduction of C-16 methyl group in addition to 9α-fluoro and double bond at the C-1, as in dexamethasone (1958) and betamethasone (1958), resulted in drugs with about 30 times the potency of hydrocortisone.

In an attempt to increase lipophilicity of corticosteroids for topical application in the 1960s and 1970s, the introduction of C-16, C-17 acetonides (Bush, 1962) and esterification at C-17 and/or C-21 hydroxyl groups (Shroot *et al.*, 1982; Ponec *et al.*, 1981) had been made. Examples include triamcinolone acetonide, betamethasone-17-valerate and betamethasone-17,21-dipropionate (1959). Other changes, more significant from the chemistry viewpoint rather than the pharmacotherapeutic viewpoint, include the replacement of the hydroxyl group at C-21 with chlorine (Phillips, 1976), Halcinonide (1962); incorporation of fused phenylpyrazole ring at C-2 and C-3, Cortivazol (1963); substitution of the 11-hydroxy group with chlorine, Meclorisone Dibutyrate (1967); incorporation of a fused oxazole ring at C-16 and C-17 (Criscuolo *et al.*, 1980), Azacort (1967) and the steroid-21-oate ester, Fluocortin Butyl (1977).

The research activities in the 1980s and early 1990s for the development of local anti-inflammatory steroids have been to increase the therapeutic index of potent corticosteroids by reducing their systemic side effects. While many of the new steroids have been obtained by extension of traditional chemical manipulation, new corticoid molecules with significant structural changes have also been developed. The novel structural modifications include the alteration of the so-called essential functional groups and the introduction of new functional groups in the glucocorticoid molecule.

In developing a new concept, several considerations have served as guidelines: (i) corticoid pharmacotherapy appears to offer an abundance of agents, but no truly safe drug, (ii) systemic manifestations of steroids are unnecessary complications which accompany treatment of many inflammatory conditions, (iii) an intact ketol chain is not an absolute requirement for the anti-inflammatory activity of corticosteroids (Schlgel, 1965; Laurent *et al.*, 1975; Soliman and Lee, 1981), and (iv) steroid acid esters with intact structures of potent glucocorticoids retain anti-in-

Table I. Hydrocortisone and its derivatives



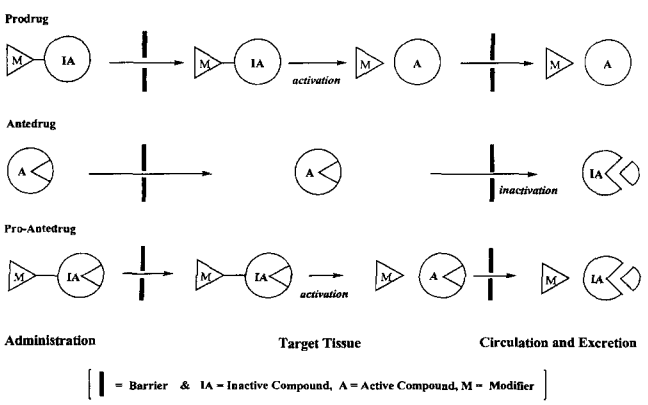
Compounds	Year introduced	Functional Groups				Activities	
		Δ^1	C6	C9	C16	Gluco.	Mineralo
Hydrocortisone	1951	-	H	H	H	1	1
Prednisolone	1955	+	H	H	H	4	0.6
Methyl Prednisolone	1956	+	CH_3	H	H	5	0
Triamcinolone	1956	+	H	F	αOH	5	0
Dexamethasone	1958	+	H	F	αOH	30	0
Betamethasone	1958	+	H	F	βOH	30	0

flammatory activity of the parent compound, but upon entry into the circulatory system from the site of administration they are hydrolyzed to steroid acids which are inactive and readily excreted (Shroot *et al.*, 1982; Lee *et al.*, 1984).

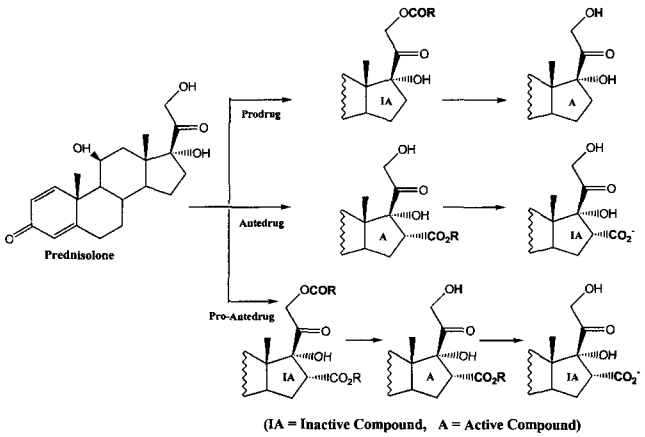
The first non-systemic anti-inflammatory steroids synthesized on the basis of a new approach were two epimers of steroid-21-oic acid ester (**1a** and **1b**). It was demonstrated that the ester derivatives of steroid-21-oic acids prepared by modifying the 17-β-ketol side chain of prednisolone retained significant local anti-inflammatory activity of the parent compound without prednisolone-like adverse systemic effects.

A new term, antedrug in contrast to the term prodrug (Albert, 1958), was introduced by Lee and his colleagues from the pharmacological profile of the steroid-21-oic acid esters (Lee and Soliman, 1982) as illustrated in scheme 1 and 2. Whereas a prodrug is an inactive compound which is activated to the active drug in a target tissue, an antedrug is an active compound at a target tissue, but is designed in such a way to undergo a predictable biotransformation to inactive metabolite which is readily excreted upon entry into systemic circulation from the applied site, Scheme 1 (Lee *et al.*, 1998). Thus, a true antedrug acts only locally and undergoes only one predictable metabolic step to an inactive metabolite. Compounds that are biotransformed to metabolites while having some degree of adverse systemic side effects can be considered as partial antedrugs; the former might include the esters of steroid-21-oic acids, and the latter might include cortisone acetate (Bodor *et al.*, 1983) and budesonide (Thalen and Brattsand, 1979).

The prodrug and the antedrug are designed drugs based on predictable metabolic activation and inactivation, respectively, as illustrated in Scheme 2. The physico-chemical properties of a drug molecule play a dominant role in determining its bioavailability. A prodrug is designed to overcome the physico-chemical limitation in the delivery of a drug into a target organ. A combination of these two properties, i.e., a designed drug to be activated



Scheme 1. Concepts of Prpdrug, Antedrug and Pro-Antedrug.



Scheme 2. Examples of Produrg, Antedrug and Pro-Antedrug.

at an intended target tissue and to be deactivated upon leaving the target tissue is defined as a pro-antedrug. On the basis of the pro-antedrug concept, Kimura *et al.* developed colonic mucosa-specific drugs for the oral treatment of ulcerative colitis (Kimura *et al.*, 1994). In a recent report, a dexamethasone-β-D-glycoside was shown to be an effective colon-specific prodrug which elicited undesirable systemic side-effects that were avoided by employing the highly polar α-D-glucopyranosyl bromide and coupling it to the methyl 1,4-pregnadien-21-oates. These hydrophilic glycosides were found to be stable in the small intestine, but the glycoside bonds were cleaved by the action of bacteria in the large intestine to release the antedrug. A common denominator of prodrug and antedrug is a transformation *in vivo*, which is either an activation (prodrug) or an inactivation (antedrug) process. The former is designed to improve the efficacy of a drug at a target tissue and the latter is designed to minimize systemic toxicities of a drug.

The following section describes synthesis and pharmacological evaluation of the steroid antedrugs. The first

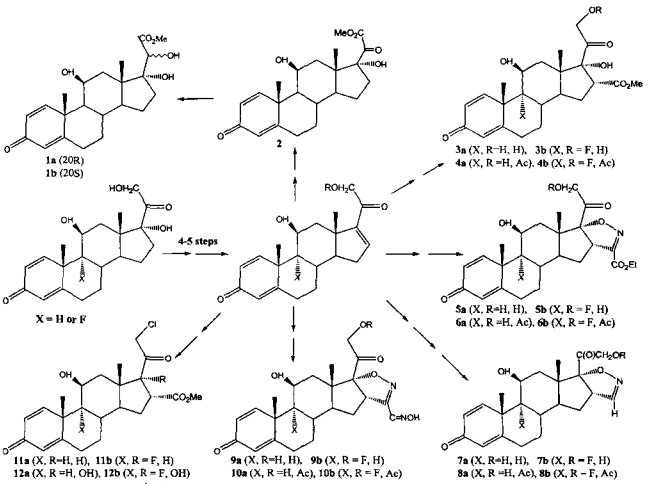


Fig. 1. Steroidal Antedrugs.

group of compounds was obtained by modifying 17β-ketol side chain; this includes steroid 20-carboxylate esters **1a,b** and **2**, Fig. 1. The second group of steroid antedrugs was obtained by introducing a metabolically labile group at carbon atom 6 or 16, this includes steroid C-16 carboxylate esters **3a,b** and **4a,b** and steroids with a heterocyclic ring such as 3-ethoxycarbonyl-[16α,17α-*d*] isoxazolinoprednisolone **5a,b** and **6a,b**, Fig. 1.

MATERIAL AND METHOD

Material

^{1}H-NMR spectra were obtained with a Brucker HX-270 spectrometer and the chemical shifts are reported in parts per million (ppm) down field from tetramethylsilane as an internal standard. Mass spectra were obtained on a Finnigan 4510 GCMS spectrometer, using positive chemical ionization. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN, and were 0.4% of the theoretical values. Melting points were determined on a Thomas Hoover Capillary Melting Point Apparatus and were uncorrected. The homogeneity of products and intermediates was monitored by TLC on Merck 60F-254 plates, with visualization under UV light. 21-Acetyloxy-9α-fluoro-11β-hydroxy-3,20-dioxo-1,4,16-pregnatriene and isoflupredone acetate were purchased from Upjohn Co. (Kalamazoo, MI). All solvents were obtained from Fisher Scientific (Orlando, FL) and were of analytical grade. All other chemicals were obtained from Aldrich Chemical Co. (Milwaukee, WI). Liquid scintillation counting was done on a 1282 Rack Compugamma counter (LKB/Wallace; Turku, Finland).

Animals

Male Sprague Dawley rats (Harlan Sprague Dawley Inc, Indianapolis, IN.) weighing 100~120 g were used. The animals were acclimatized under a controlled light/dark cycle (12 h/12 h) with constant temperature for one week prior to use and were allowed unlimited access to standard rat chow and water. Prior to the start of each experiment the animals were randomly divided into groups of five or six.

Synthesis of steroidal antedrugs: 1. steroid 20-carboxylic esters (the prototype of steroidal antedrugs)

Methyl (20R)- and (20S)-11β,17α,20-trihydroxy-3-oxo-1,4-pregnadiene-21-oate (**1a** and **1b**) were prepared by prolonged (1 week) oxidation of prednisolone **1a** with methanolic cupric acetate by applying the procedure of Lewbart and Mattox (Lewbart and Mattox, 1963) and Monder (Monder, 1971). The two epimers **1a** and **1b** were separated using semipreparative HPLC. Oxidation of prednisolone with methanolic cupric acetate for a short period (0.5 h) gave the corresponding aldehyde. Further oxidation of the aldehyde with KCN and MnO_2 in a mixture of methanol and acetic acid gave the corresponding methyl ester **2**, Methyl 11β,17α-dihy-droxy-3,20-dioxo-1,4-pregnadiene-21-oate (Lee *et al.*, 1984). This reaction is assumed to proceed through a cyanohydrin, which is then oxidized to an α-keto nitrile. The α-keto nitrile in turn is converted into an methyl ester **2** by MnO_2 in methanol.

Steroid C16-carboxylic ester antedrugs (new steroidal antedrugs)

Methyl 11β,17α,21-trihydroxy-3,20-dioxo-1,4-pregnadiene-16-carboxylate **3a** was prepared via 7 steps from **P** (Heiman *et al.*, 1997; Heiman *et al.*, 1998). **P** was treated with triethyl orthoacetate and *p*-TsOH to give cyclic orthoacetate. It was then hydrolyzed to C17-monoacetate followed by acylation of 21-alcohol to give a diacetate. Elimination of C17-acetate group gave an α,β-unsaturated enone and the subsequent one-step conversion of this enone to α-hydroxy-β-cyanoadduct afforded a C16-nitrile compound (You and Lee, 1996). Methanolysis of C16-nitrile afforded **3a** and acetylation of 21-hydroxyl group gave the compound **4a**. The compounds **3b** and **4b** were similarly obtained from 9α-fluoro-prednisolone (You *et al.*, 1995).

Fused hetereocyclo-steroidal antedrugs

A new steroid, 3'-Ethoxycarbonyl-[16α,17α-*d*] isoxazolinoprednisolone **5a**, was made by 1,3-dipolar cyclization of carbethoxyformonitrile oxide (CEFNO) to α,β-unsaturated enone (Kwon *et al.*, 1995). CEFNO was generated *in situ* by the treatment of ethyl chlorooximidoacetate with sodium bicarbonate. The 1,3-dipolar cycloaddition with fulminic acid always yielded two major products, one being the anticipated isoxazolines, **7a,b** and **8a,b** and the other hydroxyiminoformyl isoxazolines **9a,b** and **10a,b** (Khalil *et al.*, 1996; Ko *et al.*, 1997). Oximes were persistent since fulminic acid readily dimerize and the resulting dimer could add to the trienes. Though uninvited, the oximes did appear interesting due to their unique structural features and more potent biological activities. The acylation of 21-alcohol gave a corresponding acetate compound. The fluorinated steroids **5b** through **10b** were similarly obtained.

Pharmacological evaluation

Anti-inflammatory activities of the steroids were evaluated in the cotton pellet granuloma bioassay using a modification of the Meier bioassay (Lee *et al.*, 1998). Effects of topically applied steroids on edema formation were measured using the croton oil-induced ear edema bioassay (Tonneli *et al.*, 1965). Briefly, initial ear thickness of male Sprague-Dawley rats was measured with a spring-loaded micrometer. Then 25 μL of vehicle (acetone/

DMSO, 10 : 1) or sample solution containing steroid was applied to both surfaces of each rat's ears. Thirty minutes later, 25 μL of 10% solution of croton oil in acetone was applied in the same manner. The control animals were treated with only the vehicle and phlogistic agent on both ears. Five hours later at peak inflammation, ear thickness was measured. Percent inhibition of edema formation was determined by comparing the ear thickness of steroid treated animals versus control animals. The dose which inhibited ear edema by 50% (ID_{50}) was estimated from a plot of percent inhibition versus dose (μM). In five day multiple topical application studies, the drugs were applied as described above to the animals' right ears, once daily for five days, while left ears were treated with vehicle alone. Five hours following the final treatment, right and left ears were remeasured for local and systemic activities, respectively. Blood samples were obtained by cardiac puncture for plasma corticosterone measurements and relative thymus, adrenal and body weights were assessed in order to monitor adverse systemic effects of the steroids (Lee and Lee, 1985; Bird *et al.*, 1986; Heiman *et al.*, 1990; Lowry *et al.*, 1951). All data were presented as mean values of five or six samples .E.M. ANOVA, followed by least square differences between means subtest, was used to determine significant differences between groups at $p<0.05$.

RESULTS AND DISCUSSIONS

Steroid 20-carboxylic esters

The anti-inflammatory ID_{50} values (doses which inhibited inflammation by 50%), following local application in the cotton pellet granuloma and croton oil-induced ear edema model of inflammation, are shown in Table II. The results showed that the configuration of the 20-OH group might be important for anti-inflammatory potency. The compound **1b** is 5.6 times more potent than its epimer **1a** in the cotton pellet granuloma assay in the rat. While these derivatives retained considerable anti-inflammatory activity of the parent compound prednisolone, their systemic effects were greatly reduced in comparison to that of prednisolone (Lee *et al.*, 1984; Bird *et al.*, 1986). These steroids did not suppress adrenal and thymus weights or decrease plasma corticosterone level at all dose levels of 2.5 mg/cotton pellet. Methyl prednisolonate **2** showed anti-inflammatory activity in the cotton pellet granuloma bioassay comparable to that of **P** at the same dose level. Steroid **2** was devoid of any significant suppression on the thymus and adrenal weights and plasma corticosterone level (Lee *et al.*, 1984; Khalil *et al.*, 1985). Methyl prednisolonate **2** had no effect on relative adrenal weight, plasma corticosterone level or liver glycogen unlike prednisolone which significantly decreased all parameters, Table II (Olejniczak and Lee, 1984).

In a manner congruous with conventional anti-inflammatory steroids, these compounds competed against [^{3}H]-dexamethasone for binding to rat liver cytosolic receptors and inhibited leukocyte migration and prostanoid liberation at the site of inflammation (Bird *et al.*, 1986). The metabolite, 17,20-dihydroprednisolonic acid, was inactive in pharmacological tests and showed no affinity for the rat liver or thymus cytosolic glucocorticoid receptors (Lee and Lee, 1985). The effect of equipotent doses of **P** and the compounds **1a** and **1b** on the infiltration of neutrophils into saline-soaked polyester sponges was studied. Five hours after sponge implantation, **P** significantly reduced the amount of leukocyte infiltration (Bird *et al.*, 1986). This is in accordance with the proposed mechanism of action for glucocorticoids which are indirectly able to inhibit phospholipase A_2 and thereby prevent the biosynthesis of a number of pro-inflammatory mediators including prostaglandin, thromboxanes and leukocytes (Blackwell, 1983; Flower and Blackwell, 1979; Nijkamp *et al.*, 1976). Incubation of **1a** and **1b** with liver micro-

Table II. Ear edema ID_{50} values and systemic side effects from the cotton pellet bioassay

Compound	Ear Edema ID_{50}(μmol/ear)	Relative Adrenal Weight[a]	Relative Thymus Weight[a]	Relative Plasma Corticosterone[a]
P	0.54	102±7	42±7*	62±4*
1a	32.8	103±5	100±12	98±16
1b	5.9	76±6*	87±8	91±28
2	6.43	75±8*	112±10	93±19
3a	0.73	104±8	62±6	120±12
3b	0.27	101±12	93±6	75±23*
4a	0.30	104±10	93±8	131±12
4b	0.067	99±7	94±7	99±7
Acid-3b	N/A[b]	98±8	89±8	82±4

[a]Values indicate average raw values of five or six animals per group±SEM. Steroids were impregnated in right pellet (local activity) while the left pellet received only vehicle (systemic activity). ANOVA followed by a least squares differences between means subtests was used to determine values significantly different from controls at *$p<0.05$. Control mean values: adrenal weight, 17.3±1.2 mg; thymus weight, 500±22 mg.
[b]Insignificant anti-inflammatory activity at the high dose of 0.60 μmol/ear.

somal preparations led to their rapid hydrolysis (Kumari and Lee, 1985). The pharmacokinetics of **1a** and **1b** were compared to that of **P** in rats, following intramuscular administration of doses ranging from 0.5 to 10 mg/kg. The peak concentration for the three compounds at all doses was less than 5 min. The following plasma half-life values were calculated **P**: 0.53 h, **1a**: 1.2 h and **1b**: 0.28 h. From *in vitro* plasma hydrolysis studies, it was found that **1b** was completely hydrolyzed but **1a** was 30% hydrolyzed within an one hour incubation period at 35 (Al-Habet and Lee, 1990). These results suggested that lack of systemic effects may be attributable to the rapid hydrolysis of the carboxylic ester on entering the circulation.

Steroid C16-carboxylic ester compounds

New antedrugs derived from prednisolone were synthesized and the anti-inflammatory activities were evaluated. These compounds have a metabolically labile carboxylate ester at the strategic C-16 position of the steroid nucleus. Fluorination of the steroid (glucocorticoid) particularly at the 9α-position enhances all the biological activities of glucocorticoids (Phillips, 1976b; Elks, 1976). However, it is not known if fluorination of a glucocorticoid with a metabolically labile group at C16-position of prednisolone will enhance both anti-inflammatory potency and adverse systemic effects of glucocorticoids. The anti-inflammatory effects of the 16-substituted steroids **3a,b** and **4a,b** were screened in the cotton pellet granuloma bioassay (Taraporewala *et al.*, 1989; Heiman *et al.*, 1989). ID_{50} values were obtained from the dose-response data. Table II summarizes these values together with the calculated relative potencies (**p**=1.0). The results indicated that the fluorinated acetate **4b** was considerably more potent than **p**. The other esters roughly retained prednisolone's activity. The non-fluorinated esters **3a** and **4a** were examined in one group for their local/systemic anti-inflammatory activity as well as adverse systemic side effects in the cotton pellet assay and the results are summarized in Table II. The local to systemic ratio was obtained by dividing the inhibition of the granuloma formation around the steroid treated pellet by that of the untreated pellet. From this table, the acetate **4a** appears to be a true antedrug with very large local to systemic activity ratio and a lack of systemic side effects. The complete data for alcohol **3a** is not available although our preliminary studies showed a similar profile to that of **4a**. The fluorinated esters **3b** and **4b** were studied in the 5-day croton oil-induced ear edema assay for their local/systemic activity and systemic side effects. The results of this study in Fig. 2 indicate that the local to systemic activity ratio of **3b** and **4b** do not seem to offer any advantage over **p**. However, in contrast to **p**, these two steroids showed no significant alteration of body weight gain or thymus

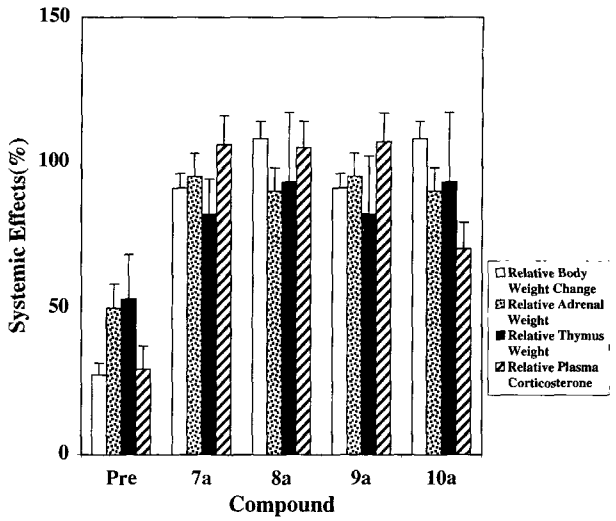


Fig. 2. Systemic side effects from the cotton pellet bioassay.

weights. As expected, the putative metabolite **FP16-acid** did not have any significant effect in multiple applications. Collectively, these results showed that the 9α-fluorination and 16α-methoxycarbonyl group, alone or in conjunction with a 21-acetate, increase topical anti-inflammatory activity without significant adverse systemicside effects.

Heterocyclic ring fused steroids

The topical anti-inflammatory activities of the compounds **5a,b** and **6a,b** were evaluated in the croton oil-induced ear edema bioassay (Khalil *et al.*, 1996; Ko *et al.*, 1997). The results in the croton oil-induced ear edema assay show that the antedrugs **5a** and its acetate **6a** retain 63% and 66% of the anti-inflammatory activity of prednisolone without showing any significant systemic activities. The fluorinated compounds **5b** and **6b** showed 4.0 and 5.4 times anti-inflammatory activity, respectively, than prednisolone. Following a single topical application in the croton oil-induced ear edema assay, all isoxazolines and oximes resulted in dose-dependent inhibition of edema. From these, ED_{50} values and relative potencies were calculated and summarized in Table III. In a semi-chronic study to determine the local to systemic anti-inflammatory activity ratio, the ED_{50} dose of numbers **7** to **10** along with **P** were topically applied to the rats' right ears followed by the phlogistic agent for five consecutive days. All compounds displayed a significant inhibition of edema in the treated right ears as shown in Table III. Significant inhibition of edema in the left ears, taken to reflect systemic activity of the absorbed steroids, was only noted for **P**. In contrast, all ring-fused steroids showed an absence of inhibition in the left ears. In the same study, systemic side effects were assessed and are also summarized in Table III. Only **P** resulted in a significant suppressive effect on normal body

Table III. Ear edema ID_{50} values and systemic side effects from the cotton pellet bioassay

Compound	Ear Edema ID_{50}(μmol/ear)	Relative Adrenal Weight[a]	Relative Thymus Weight[a]	Relative Plasma Corticosterone[b]
P	0.64	50±8*	53±15*	29±8*
7a	0.71	95±8	82±12	106±10
7b	0.33	94±7	80±5	115±14
8a	0.42	90±8	93±24	105±9
8b	0.20	106±5	101±8	98±10
9a	2.04	95±8	82±20	107±10
9b	0.16	94±7	805±	115±14
10a	0.53	90±8	93±24	70±9*
10b	0.18	106±4	101±8	67±10*

[a]Values indicate average raw values of five or six animals per group±SEM. Steroids were impregnated in right pellet (local activity) while the left pellet received only vehicle (systemic activity). ANOVA followed by a least squares differences between means subtests was used to determine values significantly different from controls at *$p<0.05$. Control mean values: adrenal weight, 28±2 mg; thymus weight, 476±27 mg.
[b]Plasma corticosterone levels. Control mean value=150±27 ng/ml.

weight gain, corticosterone level, adrenal and thymus weights. No significant suppressive effects were observed for any of the isoxazoline or oxime compounds. These results suggest that 9α-fluorination, 21-acetylation and ring fusion at C-16 and C-17 positions significantly increase anti-inflammatory potency, enhance the local/systemic activity ratio and dramatically lower adverse systemic side effects. Further pharmacological evaluations including 21-desoxy-21-chloro-16-methoxycarbonyl prednisolones and their 9-fluoro compounds, **11** and **12**, are under way.

In conclusion, the antedrug concept appears to be a fundamentally sound strategy for the development of safe and yet potent local anti-inflammatory steroids.

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