SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF CONJUGATES OF PREDNISOLONE AND NON-STEROIDAL ANTI—INFLAMMATORY AGENTS

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ABSTRACT

Esters of prednisolone with ibuprofen and indomethacin were prepared by coupling the 21-hydroxy moiety of the glucocorticoid to the carboxylic group of the non-steroidal anti-inflammatory agents. The local and systemic anti-inflammatory activities of the conjugates were evaluated using the cotton pellet granuloma bioassay and their topical activity evaluated by the croton oil-induced ear edema assay, in male Sprague-Dawley rats. The results indicate that these conjugates possess greater local and topical anti-inflammatory activity than prednisolone. In the subacute ear edema bioassay, the conjugates displayed no discernible untoward systemic effects, unlike prednisolone and prednisolone acetate, which elicited significant adverse systemic effects, at equipotent doses. These findings suggest that the chemical coupling of prednisolone and non-steroidal anti-inflammatory agents produced compounds with enhanced anti-inflammatory potencies and reduced systemic toxicities, particularly when administered topically.

INTRODUCTION

Both glucocorticoids and non-steroidal anti-inflamma-

tory agents (NSAIDs) are used to treat a variety of inflammatory conditions. A plethora of information on both classes of drugs is readily available, but there is a distinct paucity of information on the two covalently combined. Recent published reports have intimated that conjugates of glucocorticoids and anti-cancer agents exhibited greater cytolytic activity than the anti-cancer agent alone, or admixtures of the two drugs (1,2). It has also been reported that coadministration of NSAIDs with dexamethasone produced "sequential potentiation" of the anti-inflammatory activity of the glucocorticoid in the carrageenin rat paw edema assay (3).

One immediately obvious rationale for investigating conjugates of NSAIDs and glucocorticoids is that, following in vivo cleavage of the conjugates, the two components may act simultaneously and/or sequentially to inhibit steps in the inflammatory cascade, that may lend itself to increased effectiveness. This idea is somewhat akin to the sequential blockade of tetrahydrofolic acid biosynthesis by sulfamethoxazole/trimethoprim combination in antimicrobial chemotherapy. The primary objective of this study was to ascertain whether or not the chemical coupling of NSAIDs and

prednisolone would produce compounds with enhanced anti-inflammatory activity. Two such conjugates were prepared: ibuprofen-prednisolone (Ibupred), and indomethacin-prednisolone (Indopred). They were compared to prednisolone (P) and prednisolone acetate (P-Ac), in the cotton pellet granuloma and croton oil-induced ear edema assays, in terms of anti-inflammatory activity and propensity to elicit adverse systemic effects, such as suppression of plasma corticosterone levels, reduction of relative thymus and adrenal weights, and decrease in normal body weight increases.

MATERIALS AND METHODS

Chemicals and Instrumentation

Prednisolone (P) and ibuprofen were obtained from Upjohn (Kalamazoo, MI), indomethacin, croton oil and N,N'-dicyclohexylcarbodiimide (DCC) from Sigma (St. Louis, MO), methylcellulose and 4-dimethylaminopyridine (DMAP) were obtained from Aldrich Chemical Company (Milwaukee, WI). All solvents were obtained from Fisher Scientific (Orlando, FL) and were of analytical grade. Melting points were determined on a Thomas Hoover capillary melting point apparatus, and are uncorrected. Proton-NMR spectra were recorded by a Bruker HX-270 instrument and the chemical shifts are reported in parts per million (ppm) downfield from the internal standard, tetramethylsilane. Ultraviolet spectral data were generated by a Beckman 5260 spectrophotometer. Mass spectra were recorded on a Finnigan 4510 GCMS, using negative and positive chemical ionization. Infrared spectra were recorded on a Perkin-Elmer 1430 spectrophotometer, using KBr pellets. Elemental analysis samples were dried in vacuo for 48 h at 80 C (in a "drying pistol") prior to submitting to Galbraith Laboratories, Inc. (Knoxville, TN) for

analysis. The drug vehicles for local and topical applications were as follows: dichloromethane (conjugates), acetone (P), and chloroform/methanol (9:1) for P-Ac.

Synthesis of the Conjugates (Fig. 1)

Both conjugates were prepared by utilizing a modified version of the method of Neises and Steglich (4). Briefly, the non-steroidal carboxylic acids were activated by DCC and DMAP, followed by coupling of the 21-hydroxy group of the steroid (P) to the activated acids, in dry N,N-dimethylformamide (DMF). The crude products were purified by flash column chromatography, using silica-gel (Fisher 100-200 mesh, type 60 A) as the adsorbent, and chloroform/methanol (9:1 v/v) as the eluent. Homogeneity of the pure products was monitored by thin-layer chromatography (TLC) on Merck 60F-254 plates, with visualization under UV light, followed by charring with a ceric ammonium sulfate/ sulfuric acid solution.

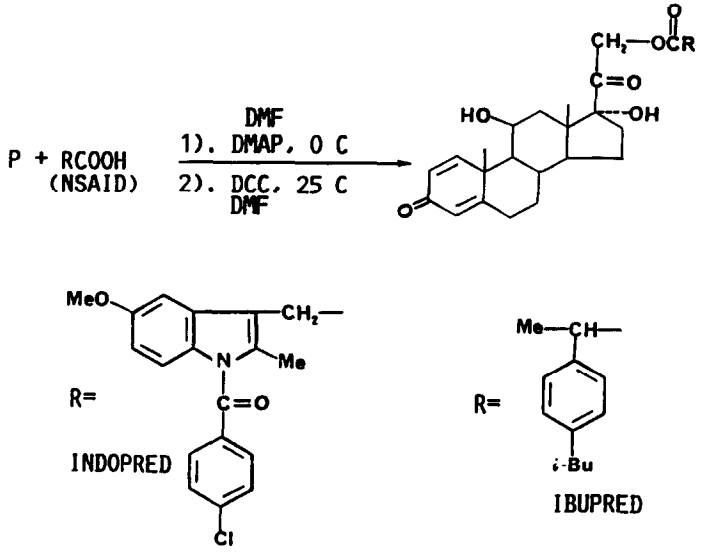

Figure 1. Synthetic scheme for the conjugates.

Ibuprofen-Prednisolone conjugate (Ibupred)

To a stirring solution of 2.06 g (10 mmol) of ibuprofen in 10 mL of dry DMF, 111 mg (0.9 mmol) of DMAP and 3.6 g (10 mmol) of P were added. After the reaction mixture was chilled to 0 C (NaCl/Ice bath), 2.27 g (11 mmol) of DCC was added and the reaction mixture stirred at room temperature for 3 h. Precipitated dicyclohexylurea (DCU) was removed by vacuum filtration and the filtrate diluted with 800 mL of dichloromethane, and washed sequentially with 0.1 N HCl (3 x 200 mL), a saturated sodium bicarbonate solution (3 x 300 mL), and distilled water (3 x 300 mL). The organic phase was dried over anhydrous sodium sulfate, followed by removal of the solvent under vacuum at 35 C, yielding an oily solid. The semi-solid was triturated with acetone/hexanes (1:1), followed by removal of solvent under vacuum, yielding an amorphous solid (4.5 g).

The crude product was subjected to flash column chromatography. The fractions containing pure product were combined, solvent removed under vacuum and the resulting solid precipitated from methylene chloride/hexanes, yielding a white amorphous solid that was homogeneous by TLC: (3 g, 54.5% yield), R_f=0.5; $CHCl_3/CH_3OH$ (9:1), 0.6; dichloromethane/acetone/hexanes (2:3:5), 0.24; hexanes/ethyl acetate (2:1) mp=180-182 C.

Proton-NMR ($CDCl_3$): ppm 0.9 (d, 6 H, 2 CH_3 of ibuprofen side chain, 1.0 (s, 3H, 13-CH_3 of P), 1.45 (s, 3H, 10-CH_3 of P), 1.55 (d, 3H, CH_3 next to carbonyl of ibuprofen), 2.45 (d, 2H, CH_2 of ibuprofen side chain), 3.85 (m, 1H, CH alpha to ester carbonyl), 4.45 (m, 1H, 11-H of P), 4.9 (m, 2H, 21-CH_2 of P), 6.01 (s, 1H, 4-H of P), 6.28 (dd, 1H, 2-H of P), 7.15 (m, 2H, phenyl protons of ibuprofen), 7.22 (m, 3H, 1-H of P and 2 phenyl protons of ibuprofen). Analyzed for C,H: Theoretical: C=74.42%; H=8.08%; Found: C=73.85%; H=8.28%. The discrepancy between theoretical and analyzed value for carbon is ascribed to persistent solvation. Mass spectral data: m/e (relative intensity) 548.28 (22.72, M^+, negative chemical ionization), 518.3 (19.26), 342.3 (100); positive chemical ionization: 549.46 (5.6, $M^+ + 1$), 207.3 (100), and infrared spectrum were consistent with the proposed structure.

Indomethacin-prednisolone conjugate (Indopred)

The above procedure was utilized for the preparation of Indopred, except that smaller quantities (2.8 mmol of P) of reactants were used. Yield: (300 mg, 16%), mp=158-160 C; R_f= 0.63; $CHCl_3/CH_3OH$ (9:1), 0.13; CH_2Cl_2/acetone/hexanes (3:2:5), 0.4; hexanes/ethyl acetate (2:1): Proton-NMR ($CDCl_3$/DMSO-d_6): ppm 0.9 (s, 3H, 13-CH_3 of P), 1.51 (s, 3H, 10-CH_3 of P), 2.35 (s, 3H, CH_3 attached to indole ring), 3.65 (s, 2H, CH_2 alpha to carbonyl of indomethacin), 3.84 (s, 3H, OCH_3 of indomethacin), 4.4 (m, 1H, 11-H of P), 4.8-5.1 (m, 2H, 21-CH_2 of P), 5.95 (s, 1H, 4-H of P), 6.20 (dd, 1H, 2-H of P), 6.65 (dd, 1H, phenyl proton of indomethacin), 6.88 (d, 1H, phenyl proton of indomethacin), 6.98 (s, 1H, phenyl proton of indomethacin), 7.21 (d, 1H, 1-H of P), 7.45 (dd, 2H, phenyl protons of indomethacin), 7.64 (dd, 2H, phenyl protons of indomethacin). Analyzed for C,H: Theoretical: C=68.61%; H=6.05%. Found: C=68.59%; H=6.72%. Discrepancy between theoretical and analyzed value for hydrogen is ascribed to persistent solvation. However, the other instrumental analysis data allowed for unambiguous structure elucidation. Mass spectral data: m/e (relative intensity): 699.2 (2.11, M^+- 1), 700.22 (1.17, M^+), 702.22 (0.42, M^++2), 357.48 (100), 342.2 (40.24), using negative chemical ionization. The infrared spectrum was also consistent with the proposed structure.

Partition Coefficients

Octanol/phosphate buffer partition coefficients were measured by a modified version of the procedure of Alhaider and co-workers (5), as previously described by Kim et al (6).

Pharmacological Evaluations

Animals: Male, Sprague-Dawley rats (Harlan Sprague-Dawley, Inc., Indianapolis, IN), weighing 100-180 g, were used throughout. The animals had unlimited access to standard rat chow and water, and were kept under a controlled light/dark cycle (12 h/12 h). Prior to the start of each experiment, the animals were randomly divided into groups of 5 or 6.

Cotton Pellet Granuloma Bioassay: Local administration of drugs: Anti-inflammatory activities of the conjugates, NSAIDs, P, and P-Ac were evaluated in the cotton pellet granuloma bioassay, using a modification of the Meier bioassay, reported by Soliman and Lee (7). Each animal received (under light ether anesthesia) a drug treated pellet, subcutaneously implanted under the right axilla, and a vehicle-treated pellet under the left axilla. Control animals were implanted with two vehicle treated pellets. Seven days later the rats were sacrificed by cervical dislocation. Inhibition of granuloma formation and systemic effects were assessed as previously described (6).

Multiple oral administration of drugs: Two untreated cotton pellets were implanted subcutaneously as described above. The test compounds were dissolved in 3 mL of ethanol, and the ethanol solutions of the drugs suspended in 10 mL of a 1% aqueous methylcellulose solution. Equimolar doses of the drugs were administered orally, once daily, for 7 days, then the animals were sacrificed and anti-inflammatory and systemic effects assessed, as previously reported (6).

Croton Oil Induced Ear Edema Assay Acute: Effects of topically applied agents on edema formation were measured, using a modified version of the croton oil-induced ear edema assay of Tonneli and co-workers (8). After initial ear thickness measurements were made with a spring loaded micrometer (Lux Scientific Instruments, NY), 25 µL of indicated amounts of steroids, dissolved in appropriate vehicles, was applied to the right ears of rats, and 25 µL of vehicle applied to the left ears. Approximately 30-60 min later, 20 µL of a 5% solution of croton oil in acetone was administered to both ears. Control animals were treated with only vehicle and phlogistic agent on both ears. All drug solutions and vehicles were applied by micropipette (Finnpipette) to the outer surface of the ears. Five h after administration of the phlogistic agent, ear thicknesses were remeasured. Percent inhibition of edema formation was determined by comparing the ear thickness of steroid treated animals to control animals. In addition, the magnitude of reduction in swelling of the contralateral ears (untreated), was used as a monitor of systemic anti-inflammatory effects of the test compounds. The dose which inhibited edema formation by 50% (ID_{50}) was estimated from

a plot of percent inhibition (right ears) versus dose (mg).

Subacute: The estimated ID_{50} doses were used in this study. The drugs were applied to the animals' right ears, and the left ears were treated with vehicle. Drug and phlogistic agent were applied topically, once daily for 7 days (9). Control animals were treated with vehicle and phlogistic agent only, during the 7-day period. Five h after the final treatment, the animals were sacrificed and anti-inflammatory and untoward systemic effects assessed as previously described.

Plasma Corticosterone Measurements: Blood samples were collected by cardiac puncture, plasma separated by centrifugation (Eppendorf centrifuge; 10 min @ 11,000 x g), and corticosterone in plasma quantitated by radioimmunoassay, utilizing the procedure in the RSL ^{3}[H]corticosterone kit (Radioassay Systems Laboratories, Inc., Carson, CA).

Statistical Analysis: One-way analysis of variance (ANOVA), followed by a least squares difference between means subtest was used to determine values significantly different from controls at $*p<0.05$.

RESULTS AND DISCUSSION

Local and systemic effects of locally administered drugs in the cotton pellet granuloma bioassay.

Anti-inflammatory activity of Ibupred, P, and ibuprofen, together with their propensity to elicit untoward systemic effects, at equimolar doses (6.9 μM), are shown in Table 1. At this dose, Ibupred displayed slightly greater local anti-inflammatory activity than P, but, unlike P, it exhibited virtually no systemic anti-inflammatory activity. In this assay, both P and Ibupred had similar degree of impact on suppression of

Table 1. LOCAL AND SYSTEMIC EFFECTS OF LOCALLY ADMINISTERED IBUPRED, P, AND IBUPROFEN IN THE COTTON PELLET GRANULOMA BIOASSAY.

Group[a]	% Inhibition[b]	Relative Thymus Weights[c] mg/100 g	Relative Adrenal Weights[c] mg/100 g	Plasma Corticosterone[c] ng/mL	Body Weight Change[c] (g)
C	---	337.5± 9.2	13.6±0.4	255.0±15.5	45.4±3.0
P	R-56.9*	167.1±18.5*	11.9±0.7*	44.5± 6.8*	42.4±1.2
	L-38.4*				
IbP	R-62.5*	105.5±16.4*	12.6±0.4	41.3± 6.4*	13.0±2.4*
	L- 8.3				
Ibu	R-10.4	306.3±18.0	14.4±0.8	225.0±52.9	35.0±3.1*
	L- 0.0				

[a]Abbreviations and doses: C=Control; P=Prednisolone (2.5 mg/right pellet; 6.9 μM), IbP=Ibupred (3.8 mg/right pellet; 6.9 μM), Ibu=Ibuprofen (1.3 mg/right pellet; 6.9 μM).
[b]Percent inhibition of granuloma formation: R=Right (treated) pellet; L=Left (untreated) pellet.
[c]Values are expressed as mean ± SEM for 5 animals: significantly different from control ($p<0.05$)*.

plasma corticosterone levels, but, unlike P, Ibupred did not significantly reduce relative adrenal weights. Ibupred caused less increases in body weights and displayed greater thymolytic effects than P, probably related to enhanced glucocorticoid effect of the conjugate. Ibuprofen displayed very weak anti-inflammatory activity, at the dose tested, and did not have

much impact on the other parameters. Anti-inflammatory and systemic effects of indopred, P, P-Ac, and indomethacin, at equimolar doses (6.9 μM), are summarized in Table 2.

Table 2. LOCAL AND SYSTEMIC EFFECTS OF LOCALLY ADMINISTERED INDOPRED, P, P-AC, AND INDOMETHACIN IN THE COTTON PELLET GRANULOMA BIOASSAY

Drug[a]	% Inhibition[b]	Relative Thymus Weights[c] mg/100 g	Relative Adrenal Weights[c] mg/100 g	Plasma Corticosterone ng/mL	Body Weight Change[c] (g)
C	---	361.9± 9.7	23.0±1.1	209.3±34.9	18.8±1.0
P	*R-64.2 *L-28.8	*146.6±18.3	*15.8±1.3	*62.0± 8.4	12.6±3.0
InP	*R-83.1 *L-64.3	*111.2± 9.8	23.8±1.3	166.0±16.5	*-13.0±2.8
Indo	*R-74.6 *L-39.1	*297.9±31.8	*19.9±0.9	*121.5±46.5	11.8±5.7
P-Ac	*R-74.6 *L-73.0	*99.5±11.3	23.5±0.3	*90.5±17.9	*-46.4±2.8

[a]Abbreviations and doses: C=Control; P=Prednisolone (2.5 mg/right pellet; 6.9 μM), InP=Indopred (4.97 mg/right pellet; 6.9 μM), Indo=Indomethacin (2.47 mg/right pellet; 6.9 μM), P-Ac=Prednisolone-21-acetate (2.8 mg/right pellet; 6.9 μM).
[b]Percent inhibition of granuloma formation: R=Right (treated) pellet; L=Left (untreated) pellet.
[c]Values are expressed as mean ± SEM for 5 animals: significantly different from control ($p<0.05$)*.

It is obvious that both the local and systemic anti-inflammatory activity of Indopred are greater than P. Furthermore, the systemic anti-inflammatory activity of Indopred is essentially a sum of that displayed by P and indomethacin, suggesting an additive effect of the two anti-inflammatory agents. Unlike P, Indopred had no impact on relative adrenal weights and caused less reduction in plasma corticosterone levels. However, Indopred inhibited normal weight gain and displayed greater thymolytic effects than P. When compared to P-Ac, it is clear that Indopred displayed superior local anti-inflammatory activity, with slightly less systemic anti-inflammatory activity, and fewer untoward effects. The local and systemic anti-inflammatory activity of indomethacin essentially mirrored that of P.

Effects of orally administered test compounds in the cotton pellet granuloma bioassay

The systemic effects of Ibupred, Indopred, P, indomethacin, and ibuprofen, following oral administration of equimolar doses (6.9 μM/kg/day), for 7 days, are depicted in Table 3. The results suggest that, in this assay, the conjugates are equipotent with P, but generally have less impact on relative thymus weights, and decreases in body weights.

Table 3. EFFECTS OF SYSTEMICALLY ADMINISTERED CONJUGATES, P, INDOMETHACIN AND IBUPROFEN, IN THE COTTON PELLET GRANULOMA BIOASSAY

Drug[a]	% Inhibition[b]	Relative Thymus Weights[c] mg/100 g	Relative Adrenal Weights[c] mg/100 g	Body Weight Change[c] (g)
C	---	221.6± 8.5	19.9±1.1	47.6±7.5
P	* 25.9	* 141.7± 7.9	17.7±0.8	* 0.0±1.9
IbP	* 23.1	* 164.1±13.4	17.8±0.8	* 10.8±2.9
InP	* 21.1	202.9±13.3	23.5±2.1	* 6.6±1.5
Ibu	11.3	* 315.7± 9.9	19.2±0.4	* 31.4±1.8
Indo	12.1	* 319.6± 8.3	17.0±1.6	45.4±1.7

[a]For abbreviations and doses: see Tables 1 and 2.
[b]Averaged percent granuloma inhibition (left and right pellet); significantly different from control ($p<0.05$)*.
[c]Values are expressed as mean ± SEM for 6 animals: significantly different from control ($p<0.05$)*.

Anti-inflammatory activity of the steroids in the acute croton oil ear edema assay

The local and systemic anti-inflammatory activities of the conjugates, P, and P-Ac, at different dose levels in the acute croton oil ear edema assay are depicted in Table 4. The conjugates displayed superior topical activity to both P and P-Ac. Even at the highest dose tested (0.729 μm/right ear), Ibupred did not display significant systemic anti-inflammatory activity,

Table 4. ACUTE CROTON OIL INDUCED EAR EDEMA ASSAY

Drug[a]	Dose µm/right ear	Mean Increase in Thickness[b] Right	Left	% Inhibition[c] Right	Left
C	---	8.2±2.0	8.4±1.0	-	-
P	0.069	6.0±1.7	10.8±0.8	26.8	-28.6
	0.693	2.8±1.1	7.4±2.5	65.9*	11.9
	1.109	1.0±0.6	4.6±1.4	87.8*	*45.2
IbP	0.009	4.6±1.2	7.4±1.0	43.9*	11.4
	0.045	4.2±0.4	9.8±1.1	48.8*	-16.7
	0.182	3.3±0.7	6.5±0.9	59.6*	22.7
	0.455	1.2±0.9	8.8±1.7	85.4*	-4.8
	0.729	1.2±1.5	9.8±2.2	85.4*	-16.7
InP	0.035	5.8±1.3	9.0±1.0	29.3	-7.1
	0.357	1.4±1.6	9.0±2.0	82.9*	-7.1
	0.571	-0.4±0.8	3.0±1.5	104.9*	*64.3
P-Ac	0.012	6.1±1.7	7.9±2.0	25.4	5.5
	0.062	6.1±1.3	7.4±2.1	25.4	11.4
	0.248	5.4±1.2	6.1±1.2	34.2	27.3

[a]For abbreviations: see Tables 1 and 2.
[b]Mean increase in thickness (mm x 10^{-2}) of right and left ears, plus or minus SEM for 5 animals.
[c]Significantly different from control: ($p<0.05$)*.

determined by reduction in swelling of the untreated contralateral ears.

It is postulated that the lack of systemic anti-inflammatory activity of Ibupred may be due to a slow rate of absorption of the conjugate into the systemic circulation, from the site of administration. Ibupred

was estimated to be 7 times more potent topically than P, and Indopred roughly 3 times as active, based on their ID_{50} values (μM), which are shown in Table 5, along with the corresponding partition coefficients and relative topical potencies.

Table 5. ESTIMATED ID_{50} VALUES, PARTITION COEFFICIENTS, AND RELATIVE TOPICAL POTENCIES OF THE STEROIDS

Drug[a]	ID_{50} (mg)	ID_{50} (μM)	Relative Potency[b] (P=1)	Partition Coefficient
P	0.17	0.47	1	23.4
IbP	0.04	0.07	6.7	30.0
InP	0.11	0.16	2.9	52.5
P-Ac	0.21	0.52	0.9	38.6

[a]For abbreviations: see Tables 1 and 2.
[b]Prednisolone was arbitrarily assigned a value of 1.

Local and systemic effects of the steroids in the subacute croton oil ear edema assay

Topical anti-inflammatory activities of P, P-Ac, and the conjugates, following multiple applications of equiactive doses, in the subacute ear edema assay are shown in Table 6. The untoward systemic effects of these compounds are summarized in Table 7.

Table 6. ANTI-INFLAMMATORY EFFECTS OF THE STEROIDS IN THE SUBACUTE CROTON OIL EAR EDEMA ASSAY

Drug[a]	Dose[b] μM/right ear	Mean Increase in Thickness[c] Right	Left	% Inhibition of Edema[d] Right	Left
C	---	11.83±1.7	14.83±1.9	--	--
P	0.47	4.70±1.1	5.33±1.1	60.6*	64.0*
IbP	0.07	1.83±0.7	14.00±1.6	84.5*	5.6
InP	0.16	5.70±0.9	8.00±1.8	52.1*	46.1*
P-Ac	0.52	8.50±1.3	11.33±1.1	28.2	23.6

[a]For abbreviations: see Tables 1 and 2.
[b]Estimated ID_{50} values; applied once daily, for 7 days.
[c]Each value is the mean (mm x 10^{-2}) ± SEM for 6 animals.
[d]Significantly different from control: ($p<0.05$)*.

The results clearly indicate that these conjugates are potent topical anti-inflammatory agents, with reduced penchant for eliciting adverse systemic effects, when compared to P and P-Ac.

The idea of coupling a steroidal anti-inflammatory agent to a NSAID is not entirely a novel one. Laurent and co-workers (10) reported the synthesis and anti-inflammatory activity of several conjugates of hydrocortisone and NSAIDs, including those with ibuprofen and indomethacin. When compared to hydrocortisone acetate, these conjugates exhibited inferior

Table 7. SYSTEMIC EFFECTS OF THE STEROIDS IN THE SUBACUTE CROTON OIL EAR EDEMA ASSAY

Drug[a]	Dose μM/right ear	Relative Thymus Weights[b] mg/100 g	Relative Adrenal Weights[b] mg/100 g	Plasma Corticosterone[b] ng/mL	Body Weight Change[b] (g)
C	---	345.4± 8.9	23.3±0.4	224.0±48.5	28.8±3.5
P	0.47	*255.2±21.1	*19.6±1.0	176.2±21.6	*21.3±1.6
IbP	0.07	341.6±10.1	21.3±1.2	211.4±24.1	30.0±0.6
InP	0.16	327.6±11.2	22.4±1.6	221.0±12.9	38.2±6.5
P-Ac	0.52	*224.5±12.4	*18.2±0.9	*153.6± 8.0	*18.3±1.7

[a]For abbreviations and doses: see Tables 1 and 2.
[b]Each value is the mean ± SEM for 6 animals; significantly different from control ($p<0.05$)*.

anti-inflammatory potency. The results obtained in the present study axiomatically establish that in the cotton pellet granuloma bioassay, these conjugates exhibited greater anti-inflammatory activity than P. They were also clearly more potent topically than both P and P-Ac.

Not only are the conjugates more potent topically, but when they were compared to P and P-Ac in the subacute croton oil ear edema assay, there were no discernible adverse systemic effects of the conjugates, whereas both P and P-Ac clearly elicited

significant untoward systemic effects. The increased topical activity of the conjugates cannot be ascribed to a simple increase in lipophilicity, judging from the partition coefficient data. For instance, both Indopred and P-Ac are more lipophilic than Ibupred, but clearly less potent. The reason for this is not immediately obvious, but it may be related to the rate at which these compounds traverse the cell membrane, and their affinities for the glucocorticoid receptor (11). Further studies on these conjugates are necessary to ascertain whether or not they are indeed superior to admixtures of steroidal and NSAIDs. Several studies are presently being designed to evaluate their binding affinity for the glucocorticoid receptor, rates of hydrolysis, and to obtain more detailed information on their systemic effects, when they are applied locally and topically.

Although few unequivocal conclusions can be drawn from these findings, it can be inferred that conjugation of prednisolone to indomethacin and ibuprofen produced compounds with enhanced local and topical anti-inflammatory activity, but which exhibit no discernible untoward systemic effect when applied topically.

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REFERENCES

1. Hong CI, Kirisits AJ, Nechaev A, Buchheit DJ and West CR (1984). Nucleoside conjugates V: Synthesis and biological activity of 9-(beta-D-Arabinofuranosyl) adenine conjugates of corticosteroids, J PHARM SCI 73:278-280.
2. An SH, West CR and Hong CI (1986). Nucleoside conjugates.8. The preparation of 5-fluoro-2'-deoxyuridine conjugates of corticosteroids. STEROIDS 47:413-420.
3. Bekemeier H and Hirschelmann R (1986). On steroid saving drug combinations in carrageenin paw edema and adjuvant arthritis. ARZNEIMITTELFORSCHUNG 36:1521-1524.
4. Neises B and Steglich W (1978). Simple method for the esterification of carboxylic acids. ANGEW CHEM INT EDIT 17:522-524.
5. Alhaider AA, Selassie CD, Chua S and Lien EJ (1982). Measurements of ionization constants and partition coefficients of guanazole prodrugs. J PHARM SCI 71:89-94.
6. Kim HP, Bird J, Heiman AS, Hudson GF, Taraporewala IB and Lee HJ (1987). Synthesis of new anti-inflammatory steroidal 20-carboxamides: (20R)-and (20S)-21-(N-substituted amino)-11 beta,17,20-trihydroxy-3,21-dioxo-1,4-pregnadiene. J MED CHEM 30:2239-2244.
7. Soliman MR and Lee HJ (1981). Local anti-inflammatory activity of acid ester derivatives of prednisolone. RES COMMUN CHEM PATHOL PHARMACOL 33:357-360.
8. Tonneli G, Thibault L and Ringler I (1965). A bioassay for the concomitant assessment of the antiphlogistic and thymolytic activities of topically applied corticoids. ENDOCRINOLOGY 77:625-634.
9. Lutsky BN, Berkenkopf J, Fernandez X, Monahan M and Watnick AS (1979). Selective effects of 7 alpha-halogeno substitution on corticosteroid ac-

tivity: Sch 22219 and Sch 23409. ARZNEIMITTELFORSCHUNG 29:992-998.
10. Laurent H, Albring M and Wendt H (1984). Synthesis and anti-inflammatory activity of esters of antiphlogistically effective carboxylic acids and hydrocortisone. ARCH PHARM (WEINHEIM) 317:421-424.
11. Mizushima Y, Igarashi R, Hoshi K, Muramatsu M and Fujita A (1986). Increase in incorporation into lymphocytes in vitro, of esterified anti-inflammatory corticosteroids. AGENTS ACTIONS 19:123-126.