SYSTEMIC ACTIVITIES OF A NEW ANTI-INFLAMMATORY STEROID: METHYL 20-DIHYDROPREDNISOLONATE

Henry J. Lee and Zulfikali M. Nathoo
Center for Anti-inflammatory Research
College of Pharmacy, Florida A&M University
Tallahassee, Florida 32307

Received 3-4-83

ABSTRACT

The systemic activities of methyl 20-dihydroprednisolonate (1), a new local anti-inflammatory steroid synthesized by modifying the 17β-ketol side-chain of prednisolone, on pituitary-adrenal function and liver glycogen content were investigated in rats. The parent compound, prednisolone, administered intramuscularly, caused a significant dose-related decrease in plasma levels of corticosterone, adrenocorticotropic hormone (ACTH), and liver glycogen content in rats. In contrast, methyl 20-dihydroprednisolonate caused mild PA suppression and liver glycogen depletion only at high doses. The steroid acid ester did not exert glycogenic activity, unlike prednisolone, in the adrenalectomized rats.

INTRODUCTION

The beneficial effects of corticosteroids in treatment of inflammatory and allergic conditions are beset by systemic adverse effects (2,3). The shortcomings are inherent in the nature of the steroids themselves; not only do glucocorticoids possess multiple biological activities, but the structural requirements for various activities appear to be overlapping and inseparable. Limited success has been made in the attempts to improve the benefit:toxicity index of these steroids by structural modifications and use of various administration methods (3). In an effort to reduce the systemic side-effects of prednisolone, methyl 20-dihydroprednisolonate was synthesized by modifying the 17β-ketol side-chain of prednisolone. Methyl 20-dihydroprednisolonate administered locally has recently been shown to retain local anti-inflammatory activity equivalent to that of the parent compound but it does not suppress pituitary-adrenal (PA) function (4,

5,6). The rationale for the synthesis of steroid-21-oate ester was based on anticipation that the steroid would possess anti-inflammatory activity similar to the parent compound when applied locally but would be hydrolyzed to inactive steroid-21-oic acid upon entry into the systemic circulation. The present investigation was undertaken to evaluate the systemic activities of the steroid acid ester on PA function and liver glycogen content.

EXPERIMENTAL

Chemicals. Prednisolone was purchased from Sigma Chemical Co. (St. Louis, Mo.). Nuclear magnetic resonance spectra were obtained on a Packard (TPC 60) spectrometer and mass spectra on AEI MS 30 using direct inlet system. Melting points were determined on Thomas capillary melting point apparatus and are uncorrected.

Methyl 20-dihydroprednisolonate was prepared according to the method of Lewbart and Mattox (7). To a solution of 6 g (17 mmole) of prednisolone in 500 ml of methanol was added a solution of 1.5 g (7.5 mmole) of copper acetate in an equal volume of methanol. The mixture was stirred for one week at room temperature with passage of air. The reaction was stopped by adding 400 ml of 1% $NaHCO_3$ containing 3 g of EDTA. After the methanol in the reaction mixture was evaporated *in vacuo*, the remaining aqueous phase was extracted with ethyl acetate. The extracts were washed with 2% $NaHCO_3, H_2O$, dried with $NaSO_4$, and the solvent was evaporated to dryness under vacuum. The steroid residue was purified with silica gel 60 column chromatography employing hexane:dichloromethane:acetone (40:20:40, v/v/v) as an eluting solvent. Crystallization of the fractions containing steroids from an acetone: hexane system gave 53% yield of epimeric mixture of the products with melting point of 138-141°C. The composition ratio of α- and β- epimers at C-20 of the product ranged 1:1 to 1:1.3 as determined by high pressure liquid chromatography. MS molecular ion (M^+) 390, NMR (δ) 1.18 (18 CH_3), 1.5 (19 CH_3), 3.78 (20 $COOCH_3$), 4.3 (11 H) *anal.* calculated for $C_{22}H_{30}O_6$: C, 67.7; H, 7.7; O, 24.6. Found C, 67.4; H, 7.9; O, 24.7.

Pharmacological Evaluation. Male albino Sprague-Dawley rats (Southern Animal Farms, Prattville, Al.) weighing 120-140 g were maintained on standard laboratory chow with water *ad libitum* under alternating light-dark cycle (lights on, 0600-1800; lights off, 1800-0600 h) and constant temperature for one week prior to their use. Rats were bilaterally adrenalectomized for measurement of glycogenic activity of steroids and maintained on normal saline for three days prior to steroid treatments.

The steroids, suspended in physiological saline:propylene glycol (1:1), were administered intramuscularly to groups of six rats for seven days at daily doses of 2.5, 5.0, and 10 mg/kg. Control animals received an equal volume of the vehicle. The blood drawn by cardiac puncture was immediately centrifuged, and the plasma was equally divided into glass and polypropylene tubes for corticosterone and ACTH determination, respectively. The excised liver was divided into one gram portions and individually wrapped in aluminum foil. The samples were stored in a freezer for subsequent analysis.

Measurement of Corticosterone, ACTH, and Glycogen. Plasma corticosterone was determined by the modified fluorometric method of Vernikos-Danellis et al. (8). Plasma ACTH was determined by radioimmunoassay using anti-ACTH serum and labeled ACTH obtained from Amersham Corp. (Arlington Heights, Il.) (9). The amount of glycogen in liver was measured by the method of Seifter et al. (10). Statistical analysis of the data was performed by one-way analysis of variance (ANOVA).

RESULTS

The systemic effects of prednisolone and its derivative on PA axis and liver glycogen content are summarized in Table. 1. Daily intramuscular injection of prednisolone for seven days caused a significant dose-related decrease in adrenal weight, plasma corticosterone, plasma ACTH, and liver glycogen at all dose levels except the adrenal weight at the 2.5 mg/kg. Administration of methyl 20-dihydroprednisolonate produced no significant change in relative adrenal weight at all doses when compared to control. Methyl 20-dihydroprednisolonate did not decrease plasma corticosterone or ACTH levels at the low doses but slightly decreased these levels at the highest dose. At a daily dose of 10.0 mg/kg, methyl 20-dihydroprednisolonate decreased plasma corticosterone and plasma ACTH by 20.1% and 18.3%, respectively. In comparison, at one-fourth of the dose level of the ester, prednisolone decreased plasma corticosterone and ACTH by 49.8% and 33.4%, respectively. A dose-related decrease in liver glycogen was observed with the ester. However, the extent of

Table 1. Effects of Systemic Administration of Steroids for One Week on Relative Adrenal Weight, Plasma Corticosterone, ACTH and Liver Glycogen

Treatment[a] (mg/kg)		Adrenal (mg/100 g Body Wt)[b]	Corticosterone (μg/100 ml)[b]	ACTH (pg/ml)[b]	Glycogen (mg/g liver)[b]
	Control	12.81±0.67	39.67±1.35	129.58±5.02	3.89±0.18
I	2.5	11.57±0.62	19.92±0.57*	86.25±5.51*	2.90±0.17*
	5.0	8.22±0.55*	13.00±0.22*	69.58±2.36*	2.01±0.09*
	10.0	7.57±0.58*	10.17±0.81*	65.00±4.03*	1.03±0.08*
II	2.5	12.68±0.67	36.43±0.88	130.85±5.61	3.35±0.26
	5.0	12.59±0.82	34.43±2.26	117.83±2.57	3.05±0.14*
	10.0	13.10±0.45	31.6 ±1.28*	105.83±2.93*	2.51±0.19*

[a]Prednisolone (I) and methyl 20-dihydroprednisolonate (II) were administered daily by intramuscular injection for seven days. The animals were sacrificed 24 hours after the last injection.

[b]Each value represents the mean ±S.E. of six separate experiments. The asterisk (*) indicates values significantly ($P < 0.05$) different from controls by one-way ANOVA.

decrease in liver glycogen by the ester was much lower than with the parent compound; the content of glycogen in liver decreased by prednisolone at the dose of 2.5 mg/kg was lower than that of the ester at 5.0 mg/kg.

Glycogenic activity of the steroids was studied in adrenalectomized animals as shown in Table 2. Adrenalectomy significantly decreased liver glycogen content (1.06 ± 0.2 mg/g liver) as compared to unadrenalectomized control liver glycogen (4.29 ± 0.42 mg/g liver). Prednisolone treatment, 5 mg/kg, for seven days, restored the liver glycogen content of adrenalectomized animals to the unadrenalectomized control values and maintained the normal increase in body weights. However, treatment with methyl 20-dihydroprednisolonate did not cause glycogen deposition in the livers of adrenalectomized rats and did not

maintain normal increases in body weights. These results indicate that the ester derivatives of prednisolone are devoid of glycogenic activity of the parent compound.

Table 2. Effects of Steroids Administered Intramuscularly on Liver Glycogen Content and Body Weights of Adrenalectomized Rats.

Treatment[a]	Liver Glycogen (mg/g Liver)[b]	Wt. of Body Change (Δ Gm)[b]
Unadrenalectomized Control	4.29±0.42	26.00±6.95
Untreated Adrenalectomized	1.06±0.2	16.17±6.17
Prednisolone	4.3 ±0.24*	35.33±4.37*
Methyl 20-dihydroprednisolone	1.63±0.21	16.67±5.02

[a]The steroids were administered by intramuscular injection for seven days at daily dose of 5 mg/kg. The animals were sacrificed 24 hours after the last injection.

[b]Each value represents the mean ± S.E. of six different experiments. Asterisk (*) indicates values ($P < 0.05$) significantly different from untreated adrenalectomized rats by one-way ANOVA.

DISCUSSION

The anti-inflammatory activity of methyl 20-dihydroprednisolonate administered locally has been shown to be equivalent to the parent compound, prednisolone, in the cotton pellet granuloma assay (3,6). However, the ester administered systemically has weaker antagonistic activity to granuloma formation than the parent compound. Furthermore, the local anti-inflammatory activity of methyl 20-dihydroprednisolonate was not accompanied by the suppression of PA function and liver glycogen depletion observed for prednisolone in intact rats (4).

Results obtained with methyl 20-dihydroprednisolonate administered systemically for seven days indicated that although at the highest dose level, 10 mg/kg, the ester caused a suppression of PA axis, the equivalent dose of the ester required for the suppression of PA

function was more than 4 times that of prednisolone. A dose-related decrease in the amount of liver glycogen was observed with the ester. However, the extent of decrease in glycogen per gram of liver observed with the ester was much less than that of prednisolone. In this study, high doses of steroids were administered deliberately in order to detect any possible systemic side-effects. In the adrenalectomized rats, the injection of prednisolone restored liver glycogen content and normal body weight gain to the level of unadrenalectomized control rats. However, methyl 20-dihydroprednisolonate faied to restore the values. The metabolite of the steroid ester, 20-dihydroprednisolonic acid, was inactive in inducing any pharmacological effects, including anti-inflammatory activity (4).

These data substantiate the hypothesis that the steroid acid esters which retain intact the ring structure of potent corticosteroids possess pharmacological activities, but upon entry into the circulatory system from the administration site are hydrolyzed to inactive steroid acids.

The C-20 carbonyl function has been considered essential for glucocorticoid activities. No anti-inflammatory steroids currently in clinical use have a reduced keto group at the C-20 position, as in methyl 20-dihydroprednisolonate. It is therefore significant that the C-20 hydroxy compound is not only an active local anti-inflammatory agent but also as potent as the C-20 keto compound prednisolone (4,6).

ACKNOWLEDGEMENTS

This research was supported by NIH Grants AM 21627 and RR 08111.

REFERENCES

1. Steroid nomenclature: prednisolone (11β,17,21-trihydroxy-1,4-pregnadiene-3,20-dione); methyl 20-dihydroprednisolonate (methyl 11β,17,20ξ-trihydroxy-3-oxo-1,4-pregnadien-21-oate).
2. Clark, J. E., and Lee, H. J. Cur. Therap. Res. 32(5), 761 (1982).
3. Soliman, M. R. I., Nathoo, Z. M., Heiman, A. S., Cook, E., and Lee, H. J. In Progress in Research and Clinical Application of Corticosteroids (ed. H. J. Lee and T. Fitzgerald), Heyden & Son, Inc., Philadelphia, (1982), pp. 253-267.
4. Lee, H. J., and Soliman, M. R. I. Science 215, 989 (1982).
5. Heiman, A. S., Nathoo, Z. M., and Lee, H. J. Drug Dev. Res. 2(4), 377 (1982).
6. Soliman, M. R. I., and Lee, H. J. Res. Comm. Chem. Path. Pharm. 33(2), 257 (1981).
7. Lewbart, M. L., and Mattox, V. R. J. Org. Chem. 28, 1773 (1963).
8. Vernikos-Danellis, J., Anderson, E., and Trigg, L. Endocrinol. 79, 624 (1966).
9. Benson, S. A., and Yalow, R. S. J. Clin. Invest. 47. 2725 (1968).
10. Seifter, S., and Dayton, S. Fed. Proc. 8, 249 (1949).