Arch. Pharm. Res. 10(3), 184-187 (1987)

Development and Structural-Activity Relationship of New Local Anti-inflammatory Steroid, Prednisolone Derivatives I. Binding Affinities to Rat Liver Glucocorticoid Receptor

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(Received August 8, 1987)

Abstract□In order to develope anti-inflammatory glucocorticoids for local use without systemic side-effects, ester and amide derivatives of 20ξ-dihydroprednisolonic acid have been prepared. When binding affinities of these compounds to glucocorticoid receptor of rat liver cytosol were compared, all α-isomer at C-20 showed higher binding affinities than the corresponding β-isomer. The size of the substituents at C-21 had significant influences on binding affinities, which were related with their lipophilicity.

Key words□Esters and amides of 20ξ-dihydroprednisolonic acid, anti-inflammatory activity, binding affinity, lipophilicity

Since the discovery of glucocorticoid, cortisone, having anti-inflammatory and anti-rheumatic activity[1], numerous structurally modified glucocorticoids including cortisone itself have been used clinically to treat the inflammatory-related and immune-disordered diseases. But extensive use of glucocorticoidal anti-inflammatory agents resulted in serious systemic side-effects such as salt-retention, P-A axis suppression and skin atrophy[2], which lead to development of chemically modified steroid with reduced side-effects. The significant advances were $\Delta^{1,2}$-dehydrogenation in prednisolone, 9α-fluorination in dexamethasone and 16β-hydroxylation in triamcinolone showing a greatly increased anti-inflammatory activity without salt-retaining mineralocorticoid effect[3]. Unfortunately, the attempts to reduce its systemic side effects excerted by glucocorticoid effects were unsuccessful mainly by the fact that anti-inflammatory activity is mediated via the same receptor to express the glucocorticoid activity. Although the many different approaches including alternate day therapy and local application of structurally modified drugs have been tried, the unwanted systemic side-effects may not be essentially avoided.

In 1982, Lee *et al.*[4] reported that methyl esters of 20ξ-dihydroprednisolonic acid **(IIa,b)** showed anti-inflammatory activity more or less than its parent compound, prednisolone **(I)**, but having a significantly reduced systemic side effects most likely through hydrolysis to inactive compounds, 20ξ-dihydroprednisolonic acid **(IIIa,b)** by esterase in the systemic circulation[5] and they proposed the "ante drug" for a compound active in the applied site but converted to inactive form in the systemic area[6]. And it was found that 20β-isomer was more potent than its corresponding 20α-isomer[7]. Further study revealed that these methyl esters **(IIa,b)** behave like conventional steroids in respects of lysosomal membrane stabilization[8], blood cell migration, prostanoids production[9] but showed no P-A axis suppression and skin atrophy[10].

From these findings, it is very interesting to study the effects on the pharmacological activity and their systemic side-effects by the different sized-substituents on C-21 position of 20ξ-dihydroprednisolonic acid and C-20 configuration. And from the same idea, it is conceivable that steroid amide at C-21 position might behave similarly as ester derivatives. Thus we have prepared the var-

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ious ester (**III-VI**) and amide derivatives (**VII-X**) of 20ξ-dihydroprednisolonic acid and compared the binding affinities presenting a important criteria for pharmacological action because these derivatives show anti-inflammatory activity by binding to glucocorticoid receptor.

In this paper, binding affinities of the various derivatives to glucocorticoid receptor depending on the substituents group and C-20 positional configuration are presented. The lipophilicity and steric effects excerted by C-21 substituents are also discussed.

EXPERIMENTAL METHODS

Meterials

Prednisolone and dexamethasone were purchased from Upjohn Co. (Kalamazoo, MI., USA). [^{3}H]-Dexamethasone (DM, 35 Ci/mmole) and Aquasol 2 scintillation fluid were obtained from New England Nuclear (Boston, Mass., USA) and all other chemicals were reagent grade purchased from Sigma Chemical Co. (St Louis, MO, USA) or Mallincroft (Paris, KY, USA).

Ester and amide derivatives of 20ξ-dihydroprednisolonic acid (Figure 1)

The detailed synthetic procedures and structural identification were previously published[11,12]. Briefly, methyl esters of 20ξ-dihydroprednisolonic acid (**IIa,b**) were prepared from prednisolone with cupric acetate in methanol according to the procedures of Kim *et al.*[11]. Separation of each isomer was achieved by preparative HPLC. From each isomer, ethyl (**IVa,b**) and propyl ester derivatives (**Va,b**) were prepared by transesterification in methanolic NaOH solution. Benzyl esters (**VIa,b**) were synthesized from each isomer of 20ξ-dihydroprednisolonic acid (**IIIa,b**) using conventional esterification procedure with sulfuric acid catalyst. Various amide derivatives (**VIIa,b-Xa,b**) were synthesized from each isomer of 20ξ-dihydroprednisolonic acid (**IIIa,b**) which were obtained by methanolic NaOH hydrolysis of methyl esters of 20ξ-dihydroprednisolonic acid (**IIa,b**), using DCC and 1-hydroxybenzotriazole, followed by treatment of respective primary amines.

Preparation of rat liver cytosol

Male Spraque-Dawley rats were bilaterally adrenalectomized and maintained on normal saline for 3-5 days prior to sacrifice. The livers were excised, perfused with cold saline and homogenized in 4 vol. of TTES buffer (10 mM Tes, 12 mM thioglycerol, 1.5 mM EDTA and 0.25 M sucrose, pH 7.4) containing 20 mM sod. molybdate using teflon pastle[13]. The homogenate was centrifuged at 105,000 g for 1 hr at 4 °C and the supernatant used as a receptor source. Protein concentrations of cytosol were determined by Lowry method[14] with bovine serum albumine as the standard.

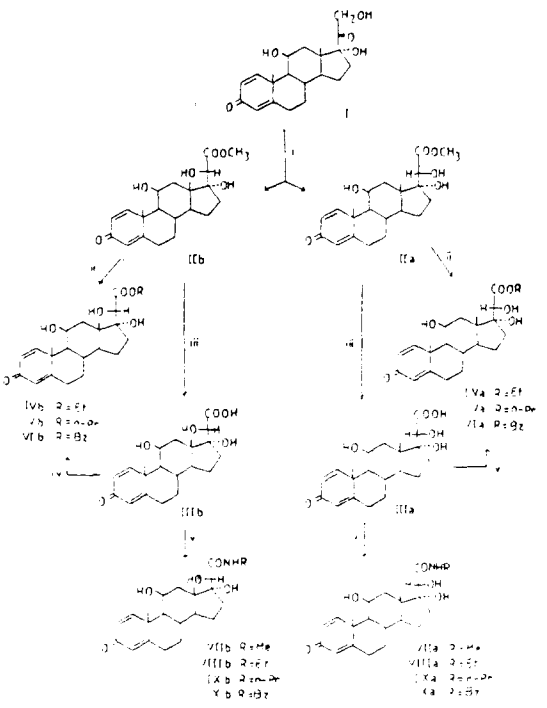

Fig. 1. Synthetic protocol of ester and carboxamide derivatives i) $Cu(OAc)_2$ in methanol, ii) OH^- in alcohol, iii) NaOH in methanol, iv) H^+, benzyl alcohol in methylene chloride, v) DCC, HOBT, followed by primary amines in methylene chloride and THF.

Steroid binding study

[^{3}H]-Dexamethasone was used as a labeled ligand for studies of the glucocorticoid binding to hepatic receptor and of the relative binding affinities of the ester and amide derivatives to this receptor. Unlabeled steroids and [^{3}H]-dexamethasone were dissolved in ethanol and aliquots dried in vacuo in incubation tubes. Following the addition of 0.1 ml of cytosol preparation, the tubes were incubated at 4 °C for 5 hrs at which time maximal binding is reached, as previously described[13]. Bound and free steroids were separated by charcoal-dextran treatment. The incubation mixture was agitated briefly on a vortex mixer after adding

Table I. Binding affinities and partition coefficients

Compounds[a]	IC_{50}[b]	Partition coefficient	Compounds	IC_{50}[b,c]	Partition[c] coefficient
Dexamethasone	32.0 nM	—[d]			
Prednisolone (I)	117.0 nM	30.0			
IIa	17.8 μM	25.1	VIIa	274.8 μM	10.0
IIb	31.0 μM	26.2	VIIb	574.0 μM	8.6
IVa	69.2 μM	27.3	VIIIa	493.2 μM	17.5
IVb	200.0 μM	30.1	VIIIb	868.9 μM	16.3
Va	74.1 μM	33.3	IXa	251.2 μM	26.7
Vb	144.5 μM	33.1	IXb	336.5 μM	16.3
VIa	11.2 μM	44.3	Xa	53.6 μM	32.5
VIb	74.1 μM	39.4	Xb	75.2 μM	30.0

[a] Steroid nomenclature: dexamethasone, 9α-fluoro-11β, 17,21-trihydroxy-16 α-methyl-pregna-1,4-dien-3,20-dione; prednisolone, 11β, 17,21-trihydroxypregna-1,4-dien-3,20-dione; IIa, IVa, Va, VIa, methyl (ethyl, n-propyl, benzyl) 11β, 17,20α -trihydroxy-3-oxopregna-1,4-dien-21-oate; IIb, IVb, Vb, VIb, methyl (ethyl, n-propyl, benzyl) 11 β, 17,20β-trihydroxy-3-oxopregna-1,4-dien-21-oate; VIIa-Xa, N-methyl (ethyl, n-propyl, benzyl) amino-11β, 17,20α-trihydroxy-3,21-dioxo-1,4-pregnadiene; VIIb-Xb, N-methyl (ethyl, n-propyl, benzyl) amino-11β, 17,20β-trihydroxy-3,21-dioxo-1,4-pregnadiene [b]Concentration required for 50% inhibition of the specific binding of 28 nM [^{3}H]-DM to hepatic receptor. [c]Data from ref. 11 [d]Not tested

0.1 ml of a suspension of 10% charcoal and 1% dextran in 10 mM Tris, pH 8.0. Following centrifugation at 3,000 g for 5 min, the supernatant (0.1 ml) was counted in 10 ml of Aquasol 2 in a Packard scintillation counter with an efficiency of ~ 30% for tritium. Quenching was corrected by the channels-ratio method. Nonspecific binding was determined by incubating 1,000-fold excess of unlabeled DM with [^{3}H]-DM and substacting from all measurements to yield specific binding. All determinations were performed in duplicate.

Measurement of 1-octanol/water partition coefficient

The partition coefficients of the various steroids in a 1-octanol-aqueous system were measured as previously described[15].

RESULTS AND DISCUSSION

Until recently, it was thought that C-20 keto and C-21 -CH_2OR in the glucocorticoids were the indispensable groups for expressing anti-inflammatory activity. But methyl esters of 20ξ-dihydroprednisolonic acid exhibited a considerable anti-inflammatory activity, furthermore, they had a greatly reduced systemic side effects[4-7]. This fact suggested that other derivatives of 20 ξ-dihydroprednisolonic acid might have similar properties.

In order to study the influences by the different substituents, we have prepared the various compounds from parent compound, prednisolone. And binding affinities in relation to lipophilicity (expressed as partition coefficient) were studied. The results were shown in Table I. Although the binding affinities of these derivatives were lower than parent compound, prednisolone, they have a significant binding affinities to the glucocorticoid receptor. Isomers having 20α-hydroxyl group showed a higher binding affinities in both series of ester and amide derivatives than the corresponding 20β-isomer. The observation of binding affinities of the derivatives in relation to the substituents size is somewhat interesting. The derivatives having smallest (methyl) and largest (benzyl) substituents in both series showed higher binding affinities than the compounds having intermediate sized-substituent (ethyl and propyl). These results indicate that binding affinities are dicreased by steric restriction when the substituents group at C-21 position in both series of derivatives become larger. But, higher binding affinities were observed in the benzyl derivatives because the high lipophilicity countervalanced the negative steric effect as previously suggested by Ponec *et al.*[16] who showed that larger substituents group in C-21 acylated cortisol led to higher binding affinity. This observation is well correlated with partition coefficient of each compound.

In conclusion, size of C-21 substituent group and the spatial arrangement of hydroxyl group at C-20 showed significant effects on the binding affinity to glucocorticoid receptor and it is suggested that these effects should be considered to develope new anti-inflammatory steroids. Anti-inflammatory activity and systemic side effects of these new derivatives are now under investigation.

ACKNOWDLEGEMENT

This research was supported by research grants from the Korea Research Foundation, the Ministry of Education, the Republic of Korea (1986).

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