Examination on Biological Activities and Fates of New Steroids, Steroid-17-yl Methyl Glycolate Derivatives

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Suzuki, T., Tada, H., Sato, E. and Tojima, Y. Examination on Biological Activities and Fates of New Steroids, Steroid-17-yl Methyl Glycolate Derivatives. Tohoku J. Exp. Med., 1999, 187 (2), 127-140 — A variety of acyl derivatives based on the “antedrug” concept were synthesized to evaluate their biological activities, in vitro fate in human serum and examine pharmacokinetics in rats. Among the prepared compounds, acetyl and pivaloyl derivatives (8 and 9) showed strong to vasoconstrictive activity in human, exceeding that of dexamethasone. In rats, topical administration of the compound 8 significantly reduced oxazolone-induced ear edema compared to that of control. These activities were almost equal to that of prednisolone, however 9 did not show any suppression of the oxazolone-induced edema. The in vitro half-lives of 8 and 9 in human serum were 18.2 and 43.8 hours, respectively. Prednisolone and dexamethasone were extremely stable under the used conditions. When compound 8 was intravenously administered to rats, its metabolites, 20(R)-methyl dexamethasone (4) and carboxylic acid (18), were found in the systemic blood. The total body clearance of 8 was 1734 ml • hr⁻¹ • kg⁻¹, which was about 12 times larger than that of dexamethasone. On the other hand, 9 was found to be metabolized instantaneously to methyl prednisolone (1) in systemic serum. Acetyl derivative 8 derived from dexamethasone may thus be useful as a topical steroid which offers the advantage of a low potential for systemic and local side effects. ——— antedrug: prednisolone; dexamethasone; corticosteroid; topical steroid © 1999 Tohoku University Medical Press

Topical steroids have been mainly used not only in atopic dermatitis, but also used in psoriasis and a number of other skin diseases. However, if potent topical steroid preparations are used for long term, or in excessive quantities, side effects will be encountered as the results of direct effect on the skin, for example, atrophy, and systemic absorption, hypotalamic-pituitary-adrenal suppression, Cushing’s...
syndrome and osteoporosis, full moon-like face due to defective lipid or protein metabolism and fatty liver and infantile growth disturbance.

Lee and Soliman (1982) proposed the "ante-drug" concept which has recently been recommended for topical steroid therapy. The term, antedrug, shows the strong pharmacological action at the site of application and when the drug enters into the systemic circulation immediately changes to the inactive form, resulting in no systemic action (Lee and Soliman 1982; Lee et al. 1984).

Prednisolonic acid esters with the intact ring structure of prednisolone were found to retain the anti-inflammatory activity of the parent compound, but following entry into the circulatory system from the site of application, to be hydrolyzed to steroid acids which are inactive and readily excreted. Although the prednisolonic acid esters exerted relatively less adverse effects, they showed milder anti-inflammatory activity than the parent compounds. Hong et al. (1989) prepared acetonides of dexamethasone acid esters based on the concept of "antedrug", however only weak anti-inflammatory activity essentially the same as that of prednisolone was observed.

An increase of lipophilia of the steroids might enhance the permeability of the drugs into epidermal layer (Scheuplein et al. 1969) and thus it might easily reach and accumulate at the inflammatory site. Therefore, acylations of methyl glycolates derived from prednisolone and dexamethasone may be an effective means of improving their permeability into the topical site and may minimize systemic side effects even in long-term use.

The purpose of the present study was to synthesize corresponding acyl derivatives of steroid-17-yl methyl glycolates, evaluate their biological activities and clarify their pharmacokinetics and in vitro behavior.

Chemistry

20$\delta$/R Steroid-17-yl methyl glycolates (1, 2), (3, 4) and each acetyl derivative (5, 6, 7 and 8) were prepared from prednisolone and dexamethasone, respectively, according to the described methods (Lewbart and Mattox 1963; Lee and Soliman 1982; Hong et al. 1989; Fujiwara et al. 1991; Suzuki et al. 1993; Kimura et al. 1994), respectively.

Pivaloyl derivatives (9, 10, 11, 12) were prepared by treatment of the corresponding methyl glycolates (1, 2, 3, 4) with pivaloyl chloride in the presence of 4-dimethylamino pyridine (DMAP), respectively.

Carboxylic acid (18) was also prepared by a treatment of the methyl glycolate (4) with 2 N potassium hydroxide in methanol according to the method used in the synthesis of the carboxylic acid (17) from the methyl glycolate (1) (Fujiwara et al. 1991).

Material and Methods

All reactions were performed under a nitrogen atmosphere. Melting points
were determined with a Yazawa BY-1 (Sendai) melting point apparatus and are uncorrected. Specific rotations were measured with a PM-101 polarimeter. \(^1\)HNMR spectra were taken for solutions in deuteriochloroform (tetramethylsilane as an internal standard) on JNM-PMX-60 (JEOL, Tokyo) and JNM-EX-270 (JEOL) instruments. IR spectra were recorded on a IR-810 (JASCO, Tokyo) spectrophotometer. Mass spectra were obtained on a JMS-OISG-2 spectrometer or JMS-BU20 Gcmate (JEOL).

Concentrations of the tested compounds in rats and human serum were determined by high pressure liquid chromatography (HPLC). The HPLC system was equipped with a 600E pump (Waters, Tokyo) and 484 Tunable Absorbance Detector (Waters). All products described in the Experimental Section were homogeneous in thin layer chromatography (TLC) and HPLC.

**Methyl(20S/R)-11β, 17α-dihydroxy-20-pivaloyloxy-3-oxo-1, 4-pregnadiene-21-oate (9 and 10).** Pivaloyl chloride (39.5 μl, 0.32 mmol) was added dropwise to a solution of 1 (50 mg, 0.13 mmol) and DMAP (47 mg, 0.38 mmol) in dry dichloromethane (3 ml) and the mixture was stirred for 22 hours at room temperature. After addition of Et₂O, the mixture was washed with small amount of water, dried (MgSO₄) and then evaporation of the solvent left a residue which was chromatographed on silica gel with CHCl₃-MeOH (99 : 1, v/v) as eluant to give 9 (59.2 mg, 97.4\%\(_0\)) as colorless caramel : \([α]_D +36.1^\circ\ (c=0.59, \text{CHCl}_3)\). IR (CHCl₃) cm\(^{-1}\): 1740, 1660 (C = O), 1620, 1605 (C = C), 1460, 1450 (C = C). \(^1\)H-NMR (270 MHz, CDCl₃) \(δ\): 1.00 (3H, s, 19-Me), 1.23 (9H, s, 19-Me), 1.43 (3H, s, 20-H), 1.57 (1H, d, \(J = 1.5\) Hz, 4-H), 6.17 (1H, d, \(J = 9, 1.5\) Hz, 2-H), 7.17 (1H, d, \(J = 9\) Hz, 1-H). HRMS \(m/z\): 474.2616 (calcd for \(\text{C}_{27}\text{H}_{38}\text{O}_7\)).

Compound 10 (43.9 mg, 89.8\%\(_0\)) was obtained as colorless caramel from 2 (50 mg) under the same condition as described above: \([α]_D +34.6^\circ\ (c=0.44, \text{CHCl}_3)\). IR (CHCl₃) cm\(^{-1}\): 1740, 1660 (C = O), 1620, 1605 (C = C), 1460, 1450 (C = C). \(^1\)H-NMR (270 MHz, CDCl₃) \(δ\): 1.02 (3H, s, 18-Me), 1.24 (9H, s, 19-Me), 1.45 (3H, s, 19-Me), 3.67 (3H, s, CO₂Me), 4.39-4.45 (1H, m, 11-H), 5.04 (1H, s, 20-H), 6.00 (1H, d, \(J = 1.5\) Hz, 4-H), 6.20 (1H, dd, \(J = 9, 1.5\) Hz, 2-H), 7.20 (1H, d, \(J = 9\) Hz, 1-H). HRMS \(m/z\): 474.2623 (calcd for \(\text{C}_{27}\text{H}_{38}\text{O}_7\)).

**Methyl(20S/R)-9α-fluoro-11β, 17α-dihydroxy-16α-methyl-20-pivaloyloxy-3-oxo-1, 4-pregnadiene-21-oate (11 and 12).** Compound 11 (59.0 mg, 98.4\%\(_0\)) was obtained as colorless syrup from 3 (50 mg) under the same condition as described above: \([α]_D +35.7^\circ\ (c=0.20, \text{CHCl}_3)\). IR (CHCl₃) cm\(^{-1}\): 1740, 1660 (C = O), 1620, 1605 (C = C), 1460, 1450 (C = C). \(^1\)H-NMR (270 MHz, CDCl₃) \(δ\): 0.82 (3H, d, \(J = 6.9\) Hz, 16-Me), 1.11 (3H, s, 18-Me), 1.26 (9H, s, 19-Me), 1.54 (3H, s, 19-Me), 3.75 (3H, s, CO₂Me), 4.32-4.40 (1H, m, 11-H), 4.93 (1H, s, 20-H), 6.11 (1H, d, \(J = 1.7\) Hz, 4-H), 6.32 (1H, dd, \(J = 10, 1.7\) Hz, 2-H), 7.18 (1H, d, \(J = 10\) Hz, 1-H). HRMS \(m/z\): 506.2681 (Calcd for \(\text{C}_{28}\text{H}_{39}\text{O}_7\) F 506.2680).
Compound 12 (38.7 mg, 80.7\%) was obtained as colorless syrup from 4 (40 mg) under the same condition as described above: $[\alpha]_D +31.1^\circ$ ($c = 0.36$, CHCl$_3$). IR (CHCl$_3$) cm$^{-1}$: 1740, 1665 (C = O), 1620, 1605 (C = C). $^1$H-NMR (270 MHz, CDCl$_3$) $\delta$: 1.13 (3H, d, $J = 6.9$ Hz, 16-Me), 1.24 (9H, s, 'Bu), 1.27 (3H, s, 18-Me), 1.54 (3H, s, 19-Me), 3.74 (3H, s, CO$_2$Me), 4.25-4.35 (1H, m, 11-H), 5.04 (1H, s, 20-H), 6.11 (1H, d, $J = 1.7$ Hz, 4-H), 6.32 (1H, dd, $J = 10$, 1.7 Hz, 2-H), 7.18 (1H, d, $J = 10$ Hz, 1-H). HRMS $m/z$: 506.2677 (calcd for C$_{28}$H$_{59}$O$_7$ F 506.2680).

**Methyl(20S / R)-11\beta, 17\alpha-dihydroxy-20-methoxycarbonyloxy-3-oxo-1, 4-pregnadiene-21-oate (13 and 14).** Methyl chloroformate (59.4 $\mu$L, 0.77 mmol) was added dropwise to a solution of 1 (100 mg, 0.26 mmol) and DMAP (94 mg, 0.77 mmol) in dry dichloromethane (2 ml) and the mixture was stirred for 10 minutes at room temperature. After quenching the reaction by an addition of water, the mixture was extracted with Et$_2$O. The extract was washed with brine, dried (MgSO$_4$) and then evaporation of the solvent left a residue which was passed though short silica gel column with CHCl$_3$ as eluant to provide 13 (114 mg, quantitative yield) as colorless syrup: $[\alpha]_D +45.7^\circ$ ($c = 0.93$, CHCl$_3$). IR (CHCl$_3$) cm$^{-1}$: 1751, 1660 (C = O), 1620, 1600 (C = C). $^1$H-NMR (270 MHz, CDCl$_3$) $\delta$: 1.07 (3H, s, 18-Me), 1.43 (3H, s, 19-Me), 3.63 (3H, s, CO$_2$Me), 3.80 (3H, s, OCO$_2$Me), 4.30-4.50 (1H, m, 11-H), 5.0 (1H, s, 20-H), 5.93 (1H, d, $J = 2$ Hz, 4-H), 6.17 (1H, dd, $J = 2$, 10 Hz, 2-H), 7.23 (1H, d, $J = 10$ Hz, 1-H). HRMS $m/z$: 448.2095 (calcd for C$_{22}$H$_{32}$O$_8$ 448.2095).

Compound 14 (112 mg, quantitative yield) was obtained as colorless syrup from 2 (100 mg) under the same condition except reaction time (2 hours) as described above: $[\alpha]_D +44.4^\circ$ ($c = 0.93$, CHCl$_3$). IR (CHCl$_3$) cm$^{-1}$: 1750, 1660 (C = O), 1620, 1600 (C = C). $^1$H-NMR (270 MHz, CDCl$_3$) $\delta$: 1.20 (3H, s, 18-Me), 1.62 (3H, s, 19-Me), 3.73 (3H, s, CO$_2$Me), 3.77 (3H, s, OCO$_2$Me), 4.27-4.50 (1H, m, 11-H), 5.0 (1H, s, 20-H), 5.97 (1H, d, $J = 2$ Hz, 4-H), 6.20 (1H, dd, $J = 10$, 2 Hz, 2-H), 7.27 (1H, d, $J = 10$ Hz, 1-H). HRMS $m/z$: 448.2096 (calcd for C$_{24}$H$_{32}$O$_8$ 448.2095).

**Methyl(20S / R)-9\alpha-fluoro-16\alpha-methyl-11\beta, 17\alpha-dihydroxy-20-methoxycarbonyloxy-3-oxo-1, 4-pregnadiene-21-oate (15 and 16).** Compound 15 (2.5 mg, 68.2\%) was obtained from 3 (3.2 mg) under the same condition (reaction time; 10 minutes) as described above: $[\alpha]_D +52.2^\circ$ ($c = 0.023$, CHCl$_3$). IR (CHCl$_3$) cm$^{-1}$: 3608, 3550-3350 (OH), 1746, 1663 (C = O), 1620, 1607 (C = C) cm$^{-1}$. $^1$H-NMR (270 MHz, CDCl$_3$) $\delta$: 0.83 (3H, d, $J = 6.9$ Hz, 16-Me), 1.12 (3H, s, 18-Me), 1.54 (3H, s, 19-Me), 3.78 (3H, s, CO$_2$Me), 3.84 (3H, s, OCO$_2$Me), 4.31-4.38 (1H, m, 11-H), 4.93 (1H, s, 20-H), 6.11 (1H, d, $J = 1.7$ Hz, 4-H), 6.33 (1H, dd, $J = 10$, 1.7 Hz, 2-H), 7.17 (1H, d, $J = 10$ Hz, 1-H). HRMS $m/z$: 448.1824 (calcd for C$_{24}$H$_{32}$O$_7$ F 448.1896).

Compound 16 (4.0 mg, 76.4\%) was obtained from 4 (4.6 mg) under the same condition (reaction time; 2 hour) as described above: $[\alpha]_D +58.3^\circ$ ($c = 0.024$,
CHCl₃. IR (CHCl₃) cm⁻¹: 3608, 3550–3350 (OH), 1750, 1660 (C = O), 1620, 1600 (C = C). ¹H-NMR (270 MHz, CDCl₃) δ: 1.10 (3H, d, J = 6.9 Hz, 16-Me), 1.27 (3H, s, 18-Me), 1.53 (3H, s, 19-Me), 3.79 (3H, s, CO₂Me), 3.82 (3H, s, OCO₂Me), 4.26–4.34 (1H, m, 11-H), 5.05 (1H, s, 20-H), 6.10 (1H, d, J = 2 Hz, 4-H), 6.32 (1H, dd, J = 10, 2 Hz, 2-H), 7.23 (1H, d, J = 10 Hz, 1-H). HRMS m/z: 480.2165 (calcd for C₂₅H₃₃O₈F 480.2160).

**Methyl(20R)-9α-fluoro-16α-methyl-11β, 17α-dihydroxy-3-oxo-1, 4-pregnadien-21-oic acid (18).** A solution of 4 (10 mg) in MeOH (0.1 ml) was treated with 2N KOH (0.05 ml) and the mixture was stirred for 2 hours at room temperature. The mixture was acidified by an addition of 10N HCl, and then extracted with chloroform. The extract was washed with brine and dried (MgSO₄). Evaporation of the solvent left a residue which was purified by preparative thin layer chromatography (silica, CHCl₃: MeOH = 3: 2 v/v) to give 18 (5.5 mg, 56.1%): [α]D +23.1° (c = 0.35, MeOH). IR (KBr) cm⁻¹: 3369 (OH), 1656 (C = O), 1600 (C = C). ¹H-NMR (CDCl₃ + CD₃OD) δ: 1.08 (d, 3H, J = 7 Hz, 16-Me), 1.29 (s, 3H, 18-Me), 1.58 (s, 3H, 19-Me), 6.06 (1H, d, J = 2 Hz, 2H), 6.27 (1H, dd, J = 10, 2 Hz, 2H), 7.37 (1H, d, J = 10 Hz, 1H). FAB-MS m/z: 408.1906 (calcd for C₂₂H₂₅FO₆ 408.1948).

**Vasoconstrictive activity in human skin.** Ten healthy volunteer subjects, male, aged between 27 and 45 years (mean = 31.5 years), weighing 55–83 kg (mean = 66.4 kg) were recruited. Volunteers gave written informed consent after receiving detailed explanation of the study protocol, including monitoring techniques, potential drug side effects, and risks related to drug administration.

Steroid derivatives were prepared in white petrolatum base (white petrolatum: liquid paraffin 9:1 w/w) at 0.1% concentration. Commercially

![Fig. 1. Structures of steroid-17-yl methyl glycolates.](image-url)
available steroidal medicaments (ointments of prednisolone, dexamethasone and budesonide) were used at 0.1% concentration in white petrolatum base as a standard. On the subject, twenty mg of the various ointments were applied to one portion in dorsal skin. The sites of application of the preparations were covered with Finn Chamber® (EPITEST Ltd., Helsinki, Finland). After each ointment was pretreated for 2 and 4 hours, it was removed pallor-index was evaluated as following: Non-effect, slight effect and moderate effects. Statistical analysis was evaluated by Kruskal-Wallis test followed by Tukey’s test.

_Half-lives in degradation of steroids and their derivatives in human serum._ Human sera were collected from five healthy volunteers (1 male and 4 females, aged between 38 and 45 years old). Each serum was pooled and immediately frozen at −80°C and stored at the same temperature until analysis. Each steroid derivative in human serum (10 mmol/0.5 ml serum) was incubated for 10, 30, 60 and 90 minutes at 37°C, respectively. Acetonitrile (0.5 ml) was added to quench the reaction and the mixture was shaken for 5 minutes. After being centrifuged for 10 minutes at 1600 × g, the supernatant was passed through a Millex-LH®, and the filtrate (100 µl) was injected into HPLC with an ultraviolet detector (245 nm). The column was µ-Bondasphere C18 (3.9 mm id × 150 mm, Waters). Acetonitrile-water (55:45, v/v) was used as mobile phase at flow rate of 0.9 ml/minutes. The degradation profile was analyzed by first order elimination kinetics.

_Anti-inflammatory activities in mouse ear edema._ Study protocol was designed based on previous article (Ptak and Asherson 1969). In practice, CBA male mice (weighing 22–28 g, Clea Japan Inc., Sendai) were used and randomly assigned to an experimental group using a table of random numbers. A volume of 0.1 ml of 3% oxazolone (2-phenyl-4-ethoxymethylene oxazolone) in alcohol was applied to the clipped abdomen as first sensitizing, and second sensitizing was carried out on 5 days later. Five days later, mice were anaesthetized with ether and the thickness of the ear was measured with an engineer’s digital micrometer. Both sides of the ear were smeared with 2% oxazolone in olive oil, and 0.5% of each tested drug in paraffin oil-DMSO (98:2 v/v) (0.02 ml) was applied to the both sides at 1 hours after the smear of oxazolone. In order to protect the ears from own physical stimulus, all mice took neck shackles (order made aluminum plate), and they were kept in animal cages one by one. The ear thickness was measured again at 24 hours after administration of the agent and the results were expressed as the increase in thickness of the ear measured in unit of 10⁻³ cm.

_Pharmacokinetics of steroid derivatives in rats._ Steroid derivatives (40 mmol/kg dose) in DMSO-isotonic sodium chloride aqueous solution (66:34 v/v) were administrated to right subclavian vein in male Spraque-Dawley strain rats (weighting 263–305 g, SLC, Shizuoka) under light ether anesthesia. Blood sam-
samples were drawn at 2, 5, 10, 30 minutes, 1, 2, 4 and 6 hours by venipuncture from left subclavian vein. Serum separated by centrifugation was immediately frozen at −80°C until HPLC analysis. After serum (50 μl) was mixed with acetonitrile (100 μl). After being centrifuged for 5 minutes at 1600×g, the supernatant (20 μl) was passed through Millex-LH® and injected into HPLC column with 20 mM phosphoric acid-acetonitrile (45 : 55 v/v) as a mobile phase.

Pharmacokinetic parameters were calculated using a two-compartment model in the nonlinear regression programs MULTI (Yamaoka et al. 1981). The estimated parameters are $Vd_{central}$, $k_{10}$ (elimination rate from central compartment), $k_{12}$ (transfer rate from compartment one to compartment two), $k_{21}$ (transfer rate from compartment two to compartment one) and $Vd_{peripheral}$. The elimination half-life ($t_{1/2}\beta$) values were calculated from ln2/$\beta$, $\beta$ was calculated by log-linear phase. $AUC_{\infty}$ is the area under the serum concentration-time curve and was calculated using the linear trapezoidal rule extrapolated to infinity. Mean residence time after bolus injection ($MRT_{\infty}$) was calculated as the area under the first moment curve ($AUMC_{\infty}$) divided by $AUC_{\infty}$. $AUMC_{\infty}$ was determined using a plot of serum concentration multiplied by time versus time and calculation of its $AUC_{\infty}$ using the trapezoidal rule extrapolated to infinity (Yamaoka et al. 1978). Total body clearance ($Cl_{total}$) is described as dose/$AUC_{\infty}$. The volume of distribution at steady-state, $Vd_{ss} = Cl_{total} \cdot AUMC_{\infty}/AUC_{\infty}$.

The protocols for animal experiments were previously approved by the Animal Research Committee, Akita University School of Medicine; all subsequent animal experiments adhered to the Guidelines for Animal Experimentation of the University.

The statistical evaluation in animal experiments was carried out by one-way analysis of variance supplemented with the multiple comparison procedure of the Scheffe in Stat View 4.5 program, and a $p$-value of <0.05 was considered to be statistical significant.

Results

The vasoconstrictive effects of synthesized compounds (1 to 16) were examined using human dorsal skin. The strength of the compounds which exhibited potent activity is shown in Figs. 2 and 3. The vasoconstrictive activity was strongest in compound 9, with the effectiveness essentially the same as budesonide. Compounds 8 and 12 expressed stronger activity than dexamethasone. The activity of 8 was weaker after 2 hours but basically the same after application for 4 hours. Compound 12 showed potent activity after application for 4 hours. The order of vasoconstrictive activity was thus shown to be $9 > 8 > 12 > 11$.

We examined the suppression effects of compounds 8 and 9 against oxazolone-induced ear edema in mice. As shown in Fig. 4, compound 8 reduced ear edema to 6.5 units (mean) in contrast to that of control. The mean suppression effect
Fig. 2. Vasoconstrictive activities of steroid derivatives at 2 hours after removal of ointment on human dorsal skin. a, Significant different at $p<0.05$, b, significant different at $p<0.01$. Key, ■; moderate vasoconstriction, □; slight vasoconstriction, A, Ointment was applied for 2 hours ($n=10$), B, Ointment was applied for 4 hours ($n=10$); DEXA, dexamethasone; BUDE, budesonide.

Fig. 3. Vasoconstrictive activities of steroid derivatives at 4 hours after removal of ointment on human dorsal skin. a, significant different at $p<0.01$. Key, ■; moderate vasoconstriction, □; slight vasoconstriction, A, Ointment was applied for 2 hours ($n=10$), B, Ointment was applied for 4 hours ($n=10$); DEXA, dexamethasone; BUDE, budesonide.
Fig. 4. Suppressive effects of compounds 8 and 9 on oxazolone induced ear edema in CBA mice. Each steroid (0.5%, 20 μl) was spread to ear edema at 1 hour after the treatment of 2% oxazolone. Ear thickness was measured at 24 hours after the oxazolone treatment. Significant difference was observed between them (*p < 0.05, **p < 0.01). The value represents the mean ± s.e. for nine to ten animals. PRED, prednisolone; DEXA, dexamethasone.

Fig. 5. The mean serum concentration-time curves of compound 8 and its metabolites after the bolus i.v. administration in rats (n = 2). Key, —○—; compound 8; —■—, methyl dexamethasone (4); —●—, the carboxylic acid (18).

was almost equal to that of prednisolone (5.6 units). Compound 9 did not show any inflammatory suppression in ear edema.

The serum concentration-time curves of compounds 8, 9 and their metabolites in rats are illustrated in Figs. 5 and 6, respectively. Compound 9 was not found in circulating serum as intact ester, while its metabolite, methyl prednisolonate (1), immediately appeared in the serum and the carboxylic acid (17) was continu-
Fig. 6. The mean serum concentration-time curves of metabolites of compound 9 after the bolus i.v. administration in rats (n=2). Key, —●--; methyl prednisoloneate (1), —■--; the carboxylic acid (17).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Configuration at C-20</th>
<th>Half-life (hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>R</td>
<td>18.2</td>
</tr>
<tr>
<td>9</td>
<td>S</td>
<td>43.8</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>—</td>
<td>N.D.</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>—</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

The mean value was obtained from the duplication. N.D., Not degraded.

Table 2. Comparison of the pharmacokinetic parameters of compound 8, dexamethasone (DEXA) and prednisolone (PRED) after the i.v. bolus administration in rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Compound</th>
<th>p value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8</td>
<td>DEXA</td>
<td>PRED</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;∞&lt;/sub&gt; (μmol × hr/ml)</td>
<td>25.5±2.95</td>
<td>358±19</td>
<td>24.3±2.3</td>
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<tr>
<td>Cl&lt;sub&gt;total&lt;/sub&gt; (ml/hr/kg)</td>
<td>1734±225</td>
<td>148±17</td>
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<td>Vd&lt;sub&gt;central&lt;/sub&gt; (ml/kg)</td>
<td>1813±104</td>
<td>427±26</td>
<td>627±44</td>
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<tr>
<td>Vd&lt;sub&gt;ss&lt;/sub&gt; (ml/kg)</td>
<td>2761±214</td>
<td>642±40</td>
<td>1962±348</td>
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<tr>
<td>Vd&lt;sub&gt;peripheral&lt;/sub&gt; (ml/kg)</td>
<td>964±281</td>
<td>219±35</td>
<td>1134±223</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2α&lt;/sub&gt; (min)</td>
<td>8.28±3.88</td>
<td>3.77±1.01</td>
<td>4.18±0.53</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2θ&lt;/sub&gt; (min)</td>
<td>94.1±20.0</td>
<td>188±23</td>
<td>42.1±6.9</td>
</tr>
<tr>
<td>k&lt;sub&gt;12&lt;/sub&gt; (hr&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>2.03±0.53</td>
<td>4.42±1.44</td>
<td>4.82±1.00</td>
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<tr>
<td>k&lt;sub&gt;21&lt;/sub&gt; (hr&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>5.03±2.19</td>
<td>9.17±3.65</td>
<td>3.06±0.42</td>
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<td>k&lt;sub&gt;10&lt;/sub&gt; (hr&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.753±0.055</td>
<td>0.353±0.048</td>
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<td>MRT&lt;sub&gt;∞&lt;/sub&gt; (hr)</td>
<td>1.61±0.09</td>
<td>4.59±0.91</td>
<td>0.952±0.249</td>
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</table>

Statistical significant was observed by multiple comparison of Scheffe based on one-way analysis. The each value represents the mean±s.e. for three animals.
ously found during the observation period. Compound 8 decreased immediately according to a two-compartment open model, and its active metabolite, methyl dexamethasone (Hong et al. 1989) (4), appeared more slowly than methyl prednisolone (1) from 9 and the carboxylic acid (18) appeared following methyl dexamethasone (4) in the systemic blood.

The mean value and statistical differences in pharmacokinetic parameters of compound 8 and reference steroids after i.v. administration in rats are shown in Table 2. The total body clearance of compound 8 was greater than that of its parent dexamethasone, $1734 \pm 255$ vs. $148 \pm 18$ ml $\cdot$ hr$^{-1} \cdot$ kg$^{-1}$, respectively ($p = 0.0040$). $V_d_{central}$ and $V_d_{ss}$ of compound 8 were significantly greater, and $t_{1/2}$ and $MRT_{\infty}$ of compound 8 were longer than those of the parent steroid, $p < 0.0001$, $p = 0.00022$, $p = 0.0289$ and $p = 0.0241$, respectively.

The in vitro half-lives of compounds 8 and 9 in human serum are shown in Table 1. They were 18.2 hours for the former and 43.3 hours for the latter. In contrast, neither prednisolone nor dexamethasone underwent degradation during the 1.5 hours of incubation in human serum.

**DISCUSSION**

Topical corticosteroids are relatively safe, however, as with any drug, they possess the risk of potential adverse reactions (Pariser 1991). This risk is high when an agent is used for long-term and has excessive potency for systemic body or skin. In the present study, we designed and synthesized new topical corticosteroid derivatives to minimize systemic side effects, and assessed their basic potential.

The test of vasoconstrictive activity has been used to assess the anti-inflammatory activity of topical corticoids, owing to its remarkably good correlation to clinical efficacy (Mckenzie and Stoughton 1962; Barry 1976). As shown in Figs. 2 and 3, the vasoconstrictive activity of dexamethasone and prednisolone were very weak on human skin. Among the tested compounds, 8 and 9 possessed potent vasoconstrictive activity on human skin; however, no suppression effect was observed at 24 hours after application of 9 for ear edema in mice, and all the samples collected following i.v. administration of compound 9 to rats were found to contain no intact ester. The absence of compound 9 in systemic serum could result from hydrolysis of the ester due to rapid metabolism in tissue and/or blood cells, and this behavior at least might result in lack of suppression of ear edema. It seems that a sufficient concentration of intact ester may be necessary in the edema for compound 9 to be sufficiently effective. But, the exact reason why compound 9 is highly active only in the dorsal skin is uncertain, since human skin would also include some esterase (Sugawara 1960). A detailed study of metabolism of compound 9 in human skin may be necessary.

We examined pharmacokinetics of compounds 8, 9 and their parent compounds by using rats. As a results, it was confirmed that kinetic parameters of
prednisolone and dexamethasone in our present study corresponded with that of the previous reports (Mulay and Varma 1984; Varma and Yue 1984; Boudinot and Jusko 1986; Garg and Jusko 1994). Compound 8 indicated total body clearance of 11.7 times of its parent dexamethasone, and its $AUC_{\infty}$ was only 7% of dexamethasone (Table 2). This suggests that compound 8 acquired a profitable characteristic as a local steroid. Total body clearance of prednisolone was about five times larger than that of dexamethasone in rats. Since compound 9 was ester derivative from prednisolone, it might be expected that 9 was metabolized extremely fast in the circulation blood.

Compound 8 expressed potent vasoconstrictive activity in humans, and the anti-inflammatory effect on oxazolone-induced ear edema in mice, and the in vivo stability was higher than that of compound 9, though the in vitro half-life of 8 in human serum was relatively short in contrast to that of 9. In the literature, hydrolysis of prednisolone in rat plasma was found to be defective (Al-Habet and Lee 1990), but normal human adult receiving single oral dose of prednisolone indicated an average half-life of 3.72 to 4.57 hours (Disanto and Desante 1975). In our study, the in vitro half-life of compounds 8 and 9 in human serum was extremely long compared to that of the whole rat body, similar to the phenomenon of hydrolysis of prednisolone in vitro. Their degradation in vitro was not correlated to that in vivo (Tables 1 and 2). These suggest that tissues, organs such as liver or kidney, and blood cell esterase may play a more important role in the metabolism of steroid 20(S/R) acyl esters than the participation of esterase in serum. Compound 8 may easily metabolize in a compartment other than serum, such as peripheral tissue (Tables 1 and 2), and in fact, both $Vd_{ss}$ and $Vd_{peripheral}$ of 8 were larger than that of its parent dexamethasone, which seems to have contributed to an increase of total body clearance and a significant reduction of the $MRT_{\infty}$ and $t_{1/2g}$ of compound 8.

It was proposed in the literature that novel local anti-inflammatory steroid should be hydrolyzed to inactive form and then to readily excreted steroid acids (Lee and Soliman 1982). As shown in Figs. 5, 6 and Table 2, because of both the efficient production of steroid acid from 8 and the large total body clearance of 8, compound 8 will result in fewer systemic effects than its parent dexamethasone.

In conclusion, this study has demonstrated that a pivaloyl derivative of methyl prednisolionate and an acetyl derivative of methyl dexamethasonate have significant vasoconstrictive activities in human dorsal skin. Acetyl derivative (8) possessed an anti-inflammatory activity in oxazolone-induced ear edema similar to that of prednisolone, and was easily metabolized to an inactive form. Therefore, compound 8 may be useful for a topical steroid owing to these characteristics.

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References


