Effects of Acute and Chronic Inflammation on the Pharmacokinetics of Prednisolone in Rats

Varun Garg,[1,2] Yuen Yi Hon,[1] and William J. Jusko[1,3]

Received August 3, 1993; accepted November 5, 1993

The effects of acute and chronic stages of carrageenan-induced air-pouch inflammation on the pharmacokinetics of prednisolone were studied in male Wistar rats. Chronic inflammation produced a significant increase in the area under the curve (AUC) of prednisolone compared to control animals (6594 ± 2144 vs 3530 ± 2164 μg · hr/L). The effect of acute inflammation was not significant (AUC = 4996 ± 3813). Both acute and chronic inflammation also reduced the *in vitro* plasma protein binding of prednisolone, the reduction being much greater after chronic inflammation. The AUC of free prednisolone after chronic inflammation was 3141 μg · hr/L, compared to 1121 μg · hr/L in the control group and 1823 μg · hr/L after acute inflammation. The mean values of half-life and apparent volume of distribution at steady-state in each group were similar. These results indicate that prednisolone must be used with caution in the treatment of inflammatory diseases because of higher free concentrations of the steroid.

KEY WORDS: prednisolone; pharmacokinetics; inflammation; protein binding.

INTRODUCTION

Prednisolone is one of the most commonly used corticosteroids in the treatment of inflammatory disorders such as rheumatoid arthritis. However, the effect of inflammatory diseases on the disposition of prednisolone or other corticosteroids has not been established. Studies in animal models of adjuvant arthritis indicate that several drug metabolizing enzymes are inhibited (1–7). Following induction of adjuvant arthritis in rats, the liver microsomal *N*-demethylase and $NADPH_2$-oxidase enzyme activity and levels of cytochrome P-450 were greatly reduced (1,2). Adjuvant arthritis also reduced the amounts of conjugated metabolites of acetaminophen recovered in the urine and decreased the maximum tolerated oral dose of phenobarbitone (1). Hexobarbital (3) or phenobarbital (4) sleeping times were also increased in such rats. These changes have been attributed to altered liver function as a result of the severe inflammatory response. However, the effect of acute inflammation on drug metabolism is less clear. Sofia (6) found that carrageenan edema, a model of acute inflammation, did not affect the hexobarbital sleeping time and zoxazolamine-induced paralysis seen with adjuvant-induced polyarthritis, a model of chronic inflammation. Other authors (8,9), however, have reported that carrageenan-induced inflammation inhibits hepatic drug metabolizing activity and decreases the cytochrome P-450 content in the male rat liver.

Besides affecting metabolism, inflammation may also affect the disposition of prednisolone by changing its plasma protein binding. Inflammation is known to cause increased plasma levels of α_1-acid glycoprotein and a number of other acute-phase proteins (10). More importantly for corticosteroids, the plasma levels of transcortin (also known as corticosteroid binding globulin; CBG) (11,12) and albumin (13) may be decreased during inflammation. There is also evidence that the inflammation causes a reduction in the affinity of CBG for corticosteroids (14). Since CBG has a high affinity and low capacity for binding prednisolone, such changes in the plasma levels and binding properties of CBG can influence the pharmacokinetics and pharmacodynamics of prednisolone.

The present study was conducted to evaluate the effects of the acute and chronic stages of inflammation on the pharmacokinetics of prednisolone. The air-pouch model of inflammation induced by carrageenan was used in this study since it simulates the inflammation occurring in synovial lining tissue and joint cavities (15). In addition, this model allows investigations in the acute exudative stage as well as the chronic proliferative stage of inflammation (16).

METHODS

Animal Experiments

Male Wistar rats weighing 300–450 g were used in this study. The rats were randomly divided into three groups of six animals each. Air-pouch inflammation was induced in rats in two of the groups by injecting 20 mL of sterile air subcutaneously in the back of the animal, followed by another 10 mL of air on the fourth day. On the seventh day, 4 mL of a 2% solution of carrageenan, warmed to 37°C, was injected into the air pouch. The third group (control) of rats was not treated with air or carrageenan. Twenty-four hours prior to administration of prednisolone, the rats in each group were anesthetized with a mixture of ketamine and xylazine administered intramuscularly and the jugular vein was cannulated. To study the effect of the acute exudative phase of inflammation, prednisolone (10 mg/kg, i.v., given as sodium succinate salt in sterile saline) was injected 6 hr after carrageenan administration. For the effect of chronic proliferative stage of inflammation, prednisolone was administered to the rats after 7 days of carrageenan administration. In all three groups, the prednisolone dose was given at the same time of day (2 P.M.). Following i.v. drug administration, the cannula was flushed with heparinized saline (20 U/mL) and blood samples were taken at preselected time points. An equal volume of blood was obtained from a donor rat and replaced. Samples were immediately centrifuged to obtain the plasma, which was stored at −20°C until analysis.

Analytical Methods

Plasma was analyzed for prednisolone, prednisone (a

[1] Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Buffalo, New York 14260.

[2] Present address: Clinical Pharmacokinetics Department, Wyeth-Ayerst Research, P.O. Box 8299, Philadelphia, Pennsylvania 19101.

[3] To whom correspondence should be addressed.

0724-8741/94/0400-0541$07.00/0

reversible metabolite of prednisolone), and corticosterone (an endogenous corticosteroid) simultaneously by high-performance liquid chromatography (17).

The *in vitro* protein binding of prednisolone was examined in the three groups by pooling the plasma from several animals in each group. For this purpose, inflammation was produced as before but no prednisolone was administered. The plasma was then spiked with prednisolone to yield concentrations in the range of 5–2000 ng/mL. Protein binding was determined in duplicate by ultrafiltration (Centrifree; Amicon, Danvers, MA) after adding trace amounts of purified (HPLC) ^{3}H-labeled prednisolone (Amersham, Arlington Heights, IL) and using a liquid scintillation counter (Packard, Downers Grove, IL) to obtain the free fraction of prednisolone.

Data Analysis

Prednisolone data were analyzed by both compartmental and noncompartmental methods to obtain the area under the plasma concentration time curve (AUC), total clearance (CL), volume of distribution at steady state (V_{ss}), and elimination half-life ($T_{1/2}$). Nonlinear curve fitting was performed using PCNONLIN (Version 4.0, Statistical Consultants Inc., Lexington, KY). Akaike's information criterion (18) was used to choose between bi- and triexponential fittings.

Prednisone AUC was calculated by the linear trapezoidal method and the ratio of prednisolone-to-prednisone AUC was calculated. Prednisolone plasma protein binding was described by the following equation (19):

$$D_B = \frac{N_T K_T P_T D_F}{1 + K_T D_F} + N_A K_A P_A D_F$$

where D_B and D_F are the molar concentrations of bound and free drug, N_T and N_A are the number of binding sites on the transcortin (T) and albumin (A) molecules, and K_T and K_A are the affinity constants. P_T and P_A are the molar concentrations of proteins in the plasma. Three protein-binding parameters (K_T, $N_T K_T P_T$, and $N_A K_A P_A$) were fitted by nonlinear regression using PCNONLIN.

The mean initial corticosterone concentrations (before prednisolone dosing) were obtained for each group. Also, the AUC of corticosterone from 0 to 45 min was evaluated in each group as a measure of suppression of corticosterone by prednisolone.

The pharmacokinetic parameters of prednisolone in rats with acute or chronic inflammation were compared with those obtained from rats in the control group using an unpaired *t* test. Significance was assessed at an alpha level of 0.05.

RESULTS AND DISCUSSION

The production of acute or chronic inflammation was confirmed in each animal by examining the air pouch for exudate production. Acute inflammation produced about 3–5 mL of exudate, while chronic inflammation produced about 5–10 mL of foul-smelling exudate. Other than the swelling at the site of inflammation, the animals appeared healthy, although the rats with chronic inflammation lost some weight (about 10–20 g per 300- to 450-g animal).

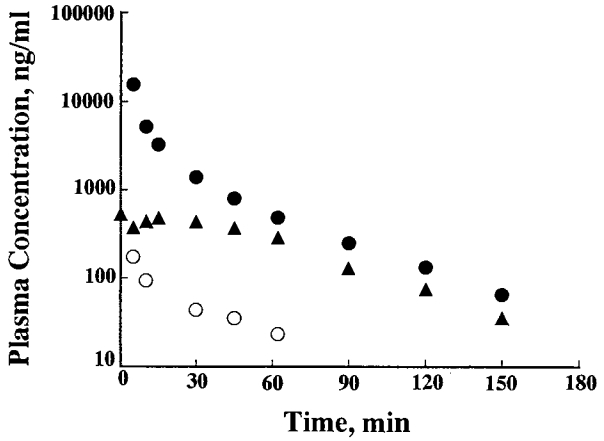

Fig. 1. Plasma concentration–time profile of prednisolone (●), prednisone (○), and corticosterone (▲) in a representative animal with acute inflammation after administration of 10 mg/kg intravenous prednisolone.

The concentration–time profile of prednisolone, prednisone, and corticosterone in a representative animal with acute inflammation is shown in Fig. 1. Prednisone and corticosterone plasma levels declined in parallel to prednisolone levels, although their concentrations were severalfold lower than the prednisolone concentrations. For many animals, corticosterone levels could be obtained at the early time points only. Therefore, only the partial AUC (0–45 min) of corticosterone was calculated. The mean prednisolone concentration–time profiles for the three groups are shown in Fig. 2. For most of the animals, a triexponential fitting was found to be optimal. However, for some animals, a biexponential equation was found to be more suitable. The mean pharmacokinetic parameters of prednisolone in the three treatment groups are summarized in Table I. The systemic clearance of prednisolone was reduced significantly in the rats in the chronic stage of inflammation, resulting in a

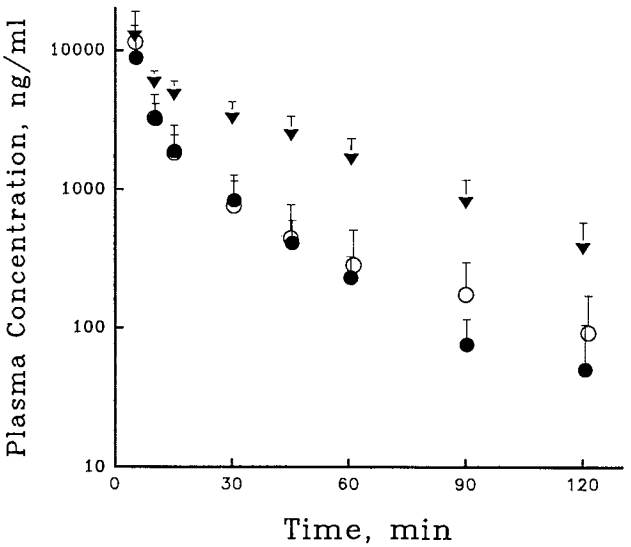

Fig. 2. Mean (±SD) plasma concentration–time profile of total prednisolone in control rats (●), rats with acute inflammation (○), and rats with chronic inflammation (▲).

Table I. Pharmacokinetic Parameters of Prednisolone in Rats Without (Control) or with Acute or Chronic Inflammation (Mean ± SD; n = 6 per group)

Parameter	Control	Acute	Chronic
AUC (μg · hr/L)	3530 ± 2164	4996 ± 3813	6594 ± 2144*
CL (L/hr/kg)	3.96 ± 2.6	3.42 ± 2.5	1.67 ± 0.59*
V_{ss} (L/kg)	1.00 ± 0.68	0.70 ± 0.61	0.51 ± 0.22
$T_{1/2}$ (hr)	0.84 ± 0.66	0.68 ± 0.39	0.51 ± 0.09
AUC PSLN/AUC PSON[a]	30.3 ± 24	—	32.8 ± 9

[a] AUC ratio of prednisolone (PSLN) to prednisone (PSON) (n = 4 in each group).
* Significantly different from control ($P < 0.05$).

higher AUC. In the rats in the acute stage of inflammation, the slightly higher AUC and lower clearance values were not significantly different from the corresponding values in the control group. The values of V_{ss} and the half-life were not significantly different across the three groups.

The ratio of AUCs of prednisolone and prednisone was about 30 in the control animals and chronic inflammation did not affect this parameter. This indicates that the metabolism of prednisone was affected to the same extent as prednisolone by chronic inflammation and that the interconversion between prednisone and prednisolone was probably not affected. In the animals with acute inflammation, the prednisone profile could be determined in only one rat, in which this ratio was 72.5.

The results of the protein binding studies are shown in Fig. 3. The protein binding parameters are presented in Table II. Chronic inflammation appears to lower the binding of prednisolone markedly, whereas acute inflammation seems to lower the binding moderately. At low concentrations, prednisolone binds mainly to CBG, which has a high affinity but low capacity for binding prednisolone. At higher concentrations, prednisolone binding to CBG is saturated and it binds mainly to albumin, which has a low affinity but high capacity for binding prednisolone. From Table II it appears that the protein binding parameter affected most by chronic inflammation was the nonspecific binding to albumin, although binding at lower steroid concentrations (to CBG) was also decreased in both acute and chronic inflammation (Fig. 3). However, statistical comparisons could not be made because the numbers represent means of two determinations from the same pools of plasma along with the standard errors associated with their estimation using PCNONLIN.

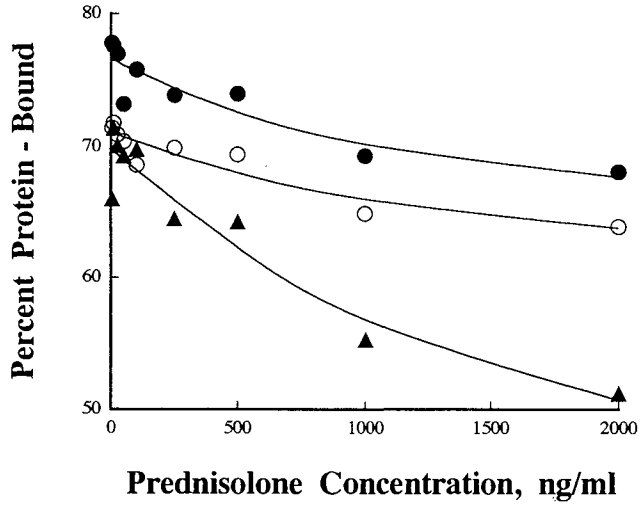

Fig. 3. Plasma protein binding of prednisolone in control rats (●), rats with acute inflammation (○), and rats with chronic inflammation (▲). Lines are drawn across fitted values.

The concentration–time profiles of free prednisolone in the three groups (not shown) were calculated using the protein-binding parameters from Table II and the mean concentration–time data presented in Fig. 2. Since chronic inflammation caused a lowering of protein binding as well as lowered clearance, the free concentrations of prednisolone were markedly higher in this group compared to the control. The AUCs of free prednisolone calculated from the mean profiles in the control, acute, and chronic groups were 1121, 1823, and 3141 μg · hr/L.

Table III shows the mean initial corticosterone concentrations and the partial AUCs in the three groups. In rats with acute inflammation, both of these parameters were significantly higher than in control animals. The values in rats with chronic inflammation were not significantly different from controls. Thus in the acutely inflamed rats, the suppressive effect of prednisolone on corticosterone plasma levels was partially offset by increased production of this endogenous corticosteroid.

The present findings are important since prednisolone is widely used in the treatment of rheumatoid arthritis and many other inflammatory diseases. It has been shown that patients with lowered prednisolone clearance are more susceptible to its side effects (20). The combined effect of lowered protein binding and reduced clearance in inflammation may lead to even more serious consequences since the free drug is generally regarded as being responsible for biological effects. Hence, studies in patients with inflammatory diseases are needed to determine if the findings in rats are clinically relevant. However, such studies are often complicated by concomitant drug therapy (e.g., nonsteroidal antiinflammatory agents) and need of suitably matched control subjects.

Table II. *In Vitro* Protein Binding Parameters of Prednisolone in Plasma of Rats Without (Control) or with Acute or Chronic Inflammation (Mean ± SE)

Parameter	Control	Acute	Chronic
$N_T P_T$ ($\times 10^7$ M)	8.5 ± 1.0	9.3 ± 0.8	1.35 ± 0.13
K_T ($\times 10^{-6}$ M^{-1})	1.8 ± 0.2	1.08 ± 0.1	1.23 ± 0.35
$N_A K_A P_A$	1.74 ± 0.07	1.44 ± 0.06	0.65 ± 0.07

Table III. Corticosterone Concentrations in the Plasma of Rats (Mean ± SD)

Parameter	Control (n = 5)	Acute (n = 5)	Chronic (n = 6)
C_0[a]	328 ± 47	480 ± 107*	271 ± 108
AUC_{0-45}[b]	7,618 ± 1,948	15,651 ± 5,252*	8,752 ± 2,182

[a] Concentration of corticosterone before prednisolone dosing.
[b] Partial AUC of corticosterone (0–45 min) after prednisolone dosing.
* Significantly different from control ($P < 0.05$).

ACKNOWLEDGMENTS

The authors thank Ms. Suzette Mis for providing technical assistance. This work was supported by Grant GM 24211 from the National Institute of General Medical Sciences, NIH.

REFERENCES

1. D. M. Morton and D. H. Chatfield. The effects of adjuvant-induced arthritis on the rat liver metabolism of drugs in rats. *Biochem. Pharmacol.* 19:473–481 (1970).
2. M. A. Cawthorne, E. D. Palmer, and J. Green. Adjuvant-induced arthritis and drug-metabolizing enzymes. *Biochem. Pharmacol.* 25:2683–2688 (1976).
3. D. M. Whitehouse. Abnormal drug metabolism in rats after an inflammatory insult. *Agents Actions* 3:312–316 (1973).
4. G. Dipasquale, P. Welaj, and C. L. Rassaert. Prolonged phenobarbital sleeping time in adjuvant-induced polyarthritic rats. *Res. Comm. Chem. Pathol. Pharmacol.* 9:253–264 (1974).
5. F. J. Beck and M. W. Whitehouse. Impaired drug metabolism in rats associated with acute inflammation: A possible assay for anti-injury agents. *Proc. Soc. Exp. Biol. Med.* 145:135–140 (1974).
6. R. D. Sofia. Alteration of hepatic microsomal enzyme systems and the lethal action of non-steroidal anti-arthritic drugs in acute and chronic models of inflammation. *Agents Actions* 7:289–297 (1977).
7. S. Ishizuki, N. Kanda, and E. Fujihara. Prostaglandins: A possible mediator to inhibit hepatic drug metabolism in adjuvant arthritic rats. *Biochem. Med. Met. Biol.* 35:40–49 (1986).
8. K. Sasaki, M. Ishikawa, Y. Takayangi, and K. Sasaki. The effect of phenobarbital on the impairment of drug metabolism by the carrageenan-induced inflammation in male rat. *Res. Comm. Chem. Pathol. Pharmacol.* 69:377–380 (1990).
9. M. Ishikawa, K. Sasaki, M. Ozaki, K. Watanabe, Y. Takayangi, and K. Sasaki. Hepatic drug metabolizing activity in rats with carrageenan-induced inflammation. *J. Pharmacobio. Dyn.* 14:132–138 (1991).
10. G. F. Fey and G. M. Fuller. Regulation of acute phase gene expression by inflammatory mediators. *Mol. Biol. Med.* 4:323–338 (1987).
11. L. Savu, C. Lombart, and E. A. Nunez. Corticosterone binding globulin: an acute phase "negative" protein in the rat. *FEBS Lett.* 113:102–106 (1980).
12. L. Bartalena, G. L. Hammond, A. Farsetti, I. L. Flink, and J. Robbins. Interleukin-6 inhibits corticosteroid-binding globulin synthesis by human hepatoblastoma-derived (Hep G2) cells. *Endocrinology* 133:291–296 (1993).
13. M. E. J. Billingham. Models of arthritis and the search for anti-arthritic drugs. *Pharmacol. Ther.* 21:389–428 (1983).
14. W. Rosner. The functions of corticosteroid-binding globulin and sex hormone-binding globulin: recent advances. *Endocr. Rev.* 11:80–91 (1990).
15. A. D. Sedgwick and P. Lees. A comparison of air-pouch, sponge and pleurisy models of acute carrageenan inflammation in the rat. *Agents Actions* 18:439–446 (1986).
16. H. Sato, M. Hashimoto, K. Sugio, K. Ohuchi, and S. Tsurufuji. Comparative study between steroidal and nonsteroidal anti-inflammatory drugs on the mode of their actions on vascular permeability in rat carrageenan-air-pouch inflammation. *J. Pharmacobio. Dyn.* 3:345–352 (1980).
17. J. Q. Rose and W. J. Jusko. Corticosteroid analysis in biological fluids by high-performance liquid chromatography. *J. Chromatogr.* 162:273–280 (1979).
18. K. Yamaoka, T. Nakagawa, and T. Uno. Application of Akaike's information criterion (AIC) in the evaluation of linear pharmacokinetic equations. *J. Pharmacok. Biopharm.* 6:165–175 (1978).
19. M. L. Rocci, N. F. Johnson, and W. J. Jusko. Serum protein binding of prednisolone in four species. *J. Pharm. Sci.* 69:977–978 (1980).
20. M. Kozower, L. Veatch, and M. M. Kaplan. Decreased clearance of prednisolone a factor in the development of corticosteroid side effects. *J. Clin. Endocrinol. Metab.* 38:407–412 (1974).