Effect of Corticosteroid Binding Globulin on the Pharmacokinetics of Prednisolone in Rats

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Purpose. The effect of exogenous corticosteroid binding globulin (CBG) on the pharmacokinetics of intravenous prednisolone was determined in rats to test the "free hormone hypothesis." ***Methods.*** A dose of CBG to yield 95% binding with 1000 ng/ml of prednisolone in vitro in rat plasma or saline was administered before dosing 2 mg/kg of prednisolone hemisuccinate or methylprednisolone intravenously. Drug concentrations in plasma samples were assayed by HPLC. ***Results.*** Single administration of CBG decreased apparent prednisolone clearance by 56% (155 to 66 ml/min/kg) and reduced apparent V_{SS} by 35% (4.1 to 2.7 L/kg) ($p<0.001$). Methylprednisolone pharmacokinetics, studied as a negative control because the drug does not bind to CBG, did not change. ***Conclusions.*** The corticosteroid bound to CBG does not appear to be available for removal by clearance organs.

KEY WORDS: corticosteroid binding globulin; transcortin; pharmacokinetics; free hormone hypothesis; prednisolone; methylprednisolone.

INTRODUCTION

Many studies have been attempted to ascertain the physiological roles of corticosteroid binding globulin (CBG) (1). One conceivable role is that CBG provides a pool of steroid in plasma to be transported into steroid-specific target cells. The most widely held thesis, the "free hormone hypothesis", states that the biological activity of steroids is determined only by the unbound concentrations in plasma (2). Baird et al. (3) proposed that the unbound and the albumin-bound fractions of steroid are transported into the liver, but not the globulin-bound steroids. Later, it was shown that the fraction bound to specific globulins is also transported into the liver (4). However, this involved use of a tissue-sampling single-injection technique which only assesses initial distribution rates. It has also been proposed that the plasma proteins increase the specificity of delivery to steroid-specific target cells by distributing the steroid toward organs with more permeable capillary beds (5).

Calculations using total versus free plasma concentrations to model prednisolone accessibility to rat liver showed no significant differences in fitting data for the dynamics of free hepatic cytosolic receptor and tyrosine aminotransferase activity (6,7). Prednisolone binds to CBG with high affinity and low capacity. The physiological level of CBG (1 μM) (8) may not be high enough in the rat to reveal the role of protein binding, if any. A maximum of 45% binding occurs and at high concentrations of prednisolone albumin is the major protein accounting for binding (9). Therefore, an elevation of CBG concentrations in the rat is required to show whether CBG alters the pharmacokinetics and pharmacodynamics of corticosteroids. This report presents the effect of CBG on the pharmacokinetics of prednisolone in rats.

METHODS

Materials

Human plasma was obtained from the American Red Cross (Buffalo, NY). EAH-Sepharose resin was purchased from Pharmacia P-L Biochemicals Inc. (Milwaukee, WI). Cortisol hemisuccinate was purchased from Steraloids Inc. (Wilton, NH). DEAE-Sephadex resin, piperazine HCl and ammonium sulphate were purchased from Sigma (St. Louis, MO). Reagents for polyacrylamide gel electrophoresis were purchased from BioRad Laboratories (Hercules, CA). The CBG RIA kit was obtained from Wien Laboratories Inc. (Succasunna, NJ). ^{3}H-prednisolone (purity 99%, specific activity 68.8 Ci/mmole) was purchased from Amersham (Arlington Heights, IL). The Centrifree system was purchased from Amicon (Beverly, MA). The other chemicals were reagent or HPLC grade.

Purification of CBG

CBG was purified by a modification of procedures reported by Kuhn et al. (10) and Fernlund and Laurell (11). The dialyzed 50-80% ammonium sulphate fraction of citrate-phosphate-dextrose treated pooled human plasma was stirred with activated charcoal (5 g/L of plasma) at room temperature for 1 hour to remove endogenous steroids, and centrifuged at 4°C for 15 min at 10,000 × g. The supernatant was filtered with Whatman no.3 paper and the filtrate was loaded into a DEAE-Sephadex column. This was washed with 2 volumes of 10 mM Tris - 5 mM $CaCl_2$, pH 7.5 at a flow rate of 50 ml/hr. Fractions of 5 ml were eluted with a linear gradient range of 0-400 mM NaCl in the buffer. All fractions were measured at 280 nm and the second peak fractions were collected. The eluate was dialyzed in 0.05 M piperazine HCl and 0.2 M NaCl buffer (pH 5.5), and then loaded into an EAH-Sepharose affinity column coupled with cortisol hemisuccinate. The column was washed at a flow rate of 50 ml/hr with the piperazine buffer until the absorbance at 280 nm was below 0.05. The column was eluted with a linear gradient of increasing cortisol in the same buffer with 10% methanol. The gradient range of cortisol was 0-1 g/L. The purity of recovered CBG was checked by 10% polyacrylamide-0.3% bis(acrylamide) gel electrophoresis according to the method of Laemmli (12).

Animals

Male Sprague-Dawley rats, weighing 200-230 g, were purchased from Harlan Sprague-Dawley Inc. (Indianapolis, IN). Animals were housed in a 12 hr light/12 hr dark, con-

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stant temperature (22°C) environment with free access to rat chow (Agway RMH 1000) and tap water ad libitum. Animals were acclimatized to this environment for at least 1 week. Rats were subject to right jugular vein cannulation under ketamine/xylazine anesthesia one day before the study.

Animal Study

A dose of CBG was determined to yield 95% binding with 1000 ng/ml of prednisolone in vitro in rat plasma. CBG or saline was administered 5 minutes before dosing 2 mg/kg of prednisolone hemisuccinate or methylprednisolone intravenously. Plasma samples of less than 0.5 ml were collected via a jugular vein catheter at times up to 180 minutes and fresh blood of sample volume from other donor rats was replenished. Three rats were used for each control and CBG treatment group for prednisolone and methylprednisolone. Plasma protein binding was determined with ^{3}H-prednisolone by ultrafiltration using the Centrifree system (13). Concentrations of CBG were measured by radioimmunoassay. Prednisolone and methylprednisolone were assayed using previously published HPLC assays (14,15).

Data Analysis

The apparent clearance (CL) and steady-state volume of distribution (V_{SS}) were calculated by moment analysis using the LAGRAN method (16). The differences in the pharmacokinetic parameters of prednisolone or methylprednisolone between control and CBG-treated rats were compared by the Student's unpaired t-test or Welche's t-test.

RESULTS

Figure 1 shows the total prednisolone concentrations in rat plasma vs. time after 2 mg/kg iv bolus doses for control and CBG-treated rats. The apparent clearances were 66 ml/min/kg with CBG and 155 ml/min/kg without CBG ($p < 0.001$). The apparent V_{SS} was 2.7 L/kg in CBG-treated rats and 4.1 L/kg in control rats ($p < 0.001$) (Figure 2). The apparent CL and V_{SS} of methylprednisolone with and without CBG treatment were not statistically significant ($p = 0.84$ and 0.32, respectively) (Figure 2). The pharmacokinetics in the control rats was consistent with previous studies in this laboratory (17,18).

Figure 3 shows that CBG concentrations declined over time but were present at sufficient concentrations so that fraction bound of prednisolone was 0.95 to 0.71 during the experiment based on the association constant of Rocci et al. (9).

DISCUSSION

In spite of various hypotheses on the roles of CBG, we could find no direct in vivo study where exogenously dosed CBG was shown to alter the disposition of corticosteroids. Similar to our present findings with prednisolone, treatment of rats with α_1-acid glycoprotein was found to reduce the clearance of prazosin (19). Corticosterone has a lower CL and V_{SS} in infant rats, which have low concentrations of CBG, but these parameters decrease concomitantly with age. The authors explained that the reduction is most likely due to an age-related increase in CBG level (20,21).

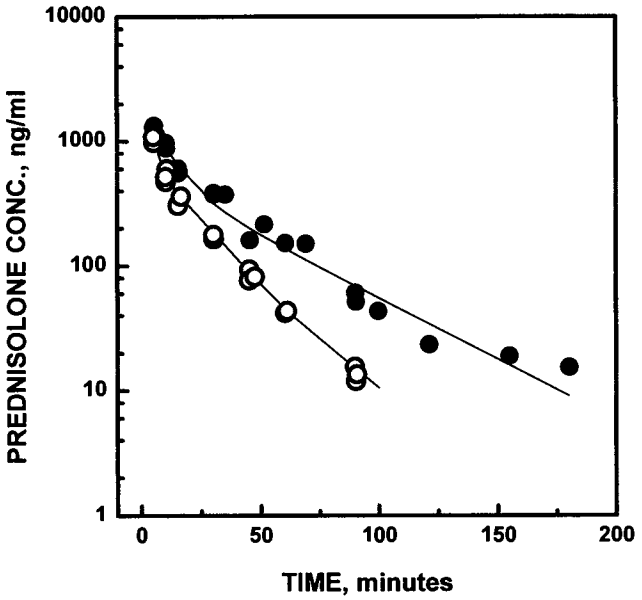

Fig. 1. Plasma concentration-time profile of prednisolone with (●) and without (○) CBG treatment after 2 mg/kg doses.

Dosing CBG in the rat should not affect osmotic pressure significantly because this would increase plasma protein concentrations by only 0.06%. An infusion of CBG would be the optimum way to maintain a constant effect of the protein, but the quantity of purified CBG was limited due to the small amount of CBG recoverable from human blood (0.72 μM)

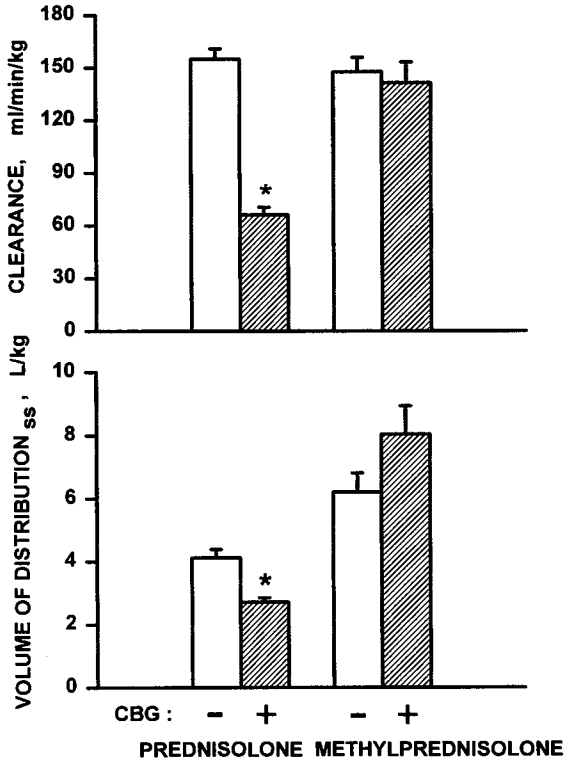

Fig. 2. Comparison of clearances and volumes of distribution (mean + SD) of prednisolone and methylprednisolone with and without CBG treatment of rats. * Statistically significant difference ($p < 0.001$).

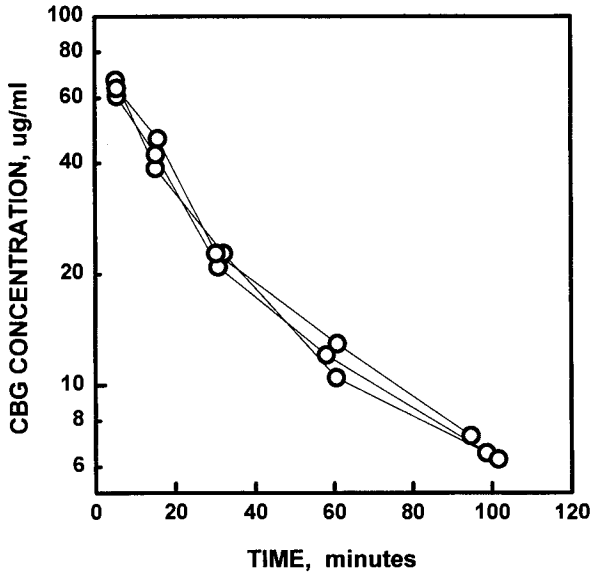

Fig. 3. Plasma concentrations of CBG versus time after intravenous administration of CBG.

(8). The dose of CBG was decided based on in vitro studies with rat plasma producing 95% binding at 1000 ng/ml of prednisolone. The calculated in vivo values of fraction bound ranged 0.95-0.71, which are higher than the normal maximum protein binding value of 0.45 in control rats (9). Since the CBG concentration and the degree of protein binding were changing over time, the calculated pharmacokinetic parameters are only approximate.

In order to determine whether other factors besides binding with exogenous CBG affect the pharmacokinetics of prednisolone, a similar experiment was carried out with methylprednisolone which does not bind to CBG (22). There was no significant difference in the apparent CL and V_{SS}. This indicates that the alteration of prednisolone pharmacokinetics is related to binding to exogenous CBG.

The results of this investigation show that increased CBG reduces the elimination of total prednisolone in plasma. This is consistent with the "free hormone hypothesis" where drug bound to some plasma proteins is not available for removal by the liver and other clearance organs. These data are also consistent with the effects of CBG on the dose-related pharmacokinetics of prednisolone in man (13,23). Higher doses of prednisolone produce increased apparent CL and V_{SS} primarily because of a smaller fraction of protein bound drug due to saturation of binding sites.

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