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International Journal of Pharmaceutics 121 (1995) 181–186

international journal of pharmaceutics

# Effect of acetazolamide on the pharmacokinetics of cyclosporin in rabbits

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Received 26 September 1994; accepted 20 December 1994

**Abstract**

The effect of concomitant intravenous administration of acetazolamide on cyclosporin's pharmacokinetic parameters has been studied in rabbits. The study design was parallel: the control group of six rabbits received cyclosporin alone, while cyclosporin and acetazolamide were co-administered to six rabbits in the second group. Serial blood samples were collected for 24 h following administration of drugs. Cyclosporin concentrations were determined using a specific monoclonal radioimmunoassay. Pharmacokinetic parameters of cyclosporin were derived using compartmental and non-compartmental techniques. Concomitant administration of acetazolamide with cyclosporin resulted in a significant increase in the terminal elimination half-life of cyclosporin, a significant decrease in cyclosporin total body clearance, and a significant increase in the steady-state volume of distribution relative to the control group. The mean residence time of cyclosporin in the peripheral tissue was dramatically increased with the administration of acetazolamide. The results of this investigation demonstrate a possible interaction between acetazolamide and cyclosporin. The most plausible explanation for this is an alteration in the disposition kinetics of cyclosporin. Adjusting the dose of cyclosporin may be required in patients with uveitis receiving both cyclosporin and acetazolamide.

*Keywords:* Cyclosporin; Acetazolamide; Pharmacokinetic parameters

## 1. Introduction

Cyclosporin, a neutral lipophilic cyclic polypeptide with unique immunosuppressive properties, has been extensively used in transplant recipients (Freeman, 1991; Faulds et al., 1993). More recently, cyclosporin has been evaluated for use in a variety of other immune-mediated disorders (Moolman et al., 1991; Chavis et al., 1992; Christophers et al., 1992; Schrezenmeier et al., 1992; Sherman and Pinto, 1992). Following intravenous administration, cyclosporin is rapidly (within about 10 min) distributed between blood cells (60–70%) and plasma. Most of the cyclosporin in blood cells is taken up by erythrocytes (41–58%), whereas most of the drug in plasma is bound to lipoproteins (34%) (Kahan,

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*SSDI* 0378-5173(95)00012-7

1989; Gupta and Benet, 1990). Subsequently, the majority of the cyclosporin dose is distributed outside the blood with a large apparent volume of distribution (Fahr, 1993). Cyclosporin is extensively (99%) metabolized in the liver, exhibits linear elimination kinetics and is considered a drug of low-to-intermediate clearance (Fahr, 1993).

Acetazolamide, a heterocyclic sulfonamide, acts as a noncompetitive inhibitor of the enzyme carbonic anhydrase. Its action on the renal tubule results in a bicarbonate diuresis and a mild self-limiting metabolic acidosis (Gilman et al., 1985). The presence of carbonic anhydrase in a number of intraocular structures, including the ciliary processes, and the high concentrations of bicarbonate in the aqueous humor were the factors that called attention to the role that the enzyme and its inhibitor might play in the production of aqueous humor. Acetazolamide reduces the rate of aqueous humor formation, leading to a decrease in intraocular pressure in patients with glaucoma (Gilman et al., 1985).

Acetazolamide is 70–90% protein bound and is widely distributed throughout body tissues, especially those with high carbonic anhydrase concentrations (renal cortex and red blood cells) (Bennett et al., 1987). The drug is not metabolized in the body and mainly eliminated unchanged in urine (Bennett et al., 1987).

When administered in conjunction with cyclosporin, acetazolamide has been shown to cause a 5-fold increase in cyclosporin trough serum levels as well as pronounced nephrotoxicity and neurotoxicity (Keogh et al., 1988).

Cyclosporin has been used widely in the treatment of many forms of uveitis. Since intraocular pressure can increase in patients with uveitis and cause secondary glaucoma, it is sometimes necessary to use cyclosporin concomitantly with acetazolamide to control the glaucoma. One of the authors of the present study noted an increase in the serum level of cyclosporin following the co-administration of acetazolamide in a patient with uveitis. We therefore decided to study the effect of concomitant intravenous administration of acetazolamide and cyclosporin on cyclosporin's pharmacokinetic parameters in rabbits.

## 2. Materials and methods

### 2.1. Chemicals

Cyclosporin (Sandimmun®, 250 mg/5 ml) was obtained from Sandoz Ltd (Basel, Switzerland). Acetazolamide in vials (Diamox®, 500 mg/10 ml) was obtained from Lederle Laboratories (American Cyanamid Co., NY, USA).

### 2.2. Drug administration and sampling

New Zealand white male rabbits, weighing from 3.0 to 4.3 kg, were used. The animals were fasted for 12 h before the experiment began as well as during the experiment, although water was allowed ad libitum. The animals were immobilized in a restraining box when drugs were administered and when blood samples were taken.

The study design was parallel; the acetazolamide-treated group and the control group each comprised six rabbits. The dose of cyclosporin (15 mg/kg) was mixed with normal saline (0.9%) and administered as an i.v. bolus over a period of 3–4 min in the marginal ear vein. Immediately following cyclosporin administration, six rabbits received an intravenous dose (10 mg/kg) of acetazolamide in the marginal vein of the opposite ear. The marginal vein of one ear was cannulated with a polyethylene tube (Terumo 22 G) for blood sampling. Multiple blood samples (1.5 ml) were collected in evacuated glass tubes containing EDTA anticoagulant at 0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0 and 24.0 h following administration of the drugs. All samples were then refrigerated (4° C) until analysis, which was performed within 1 week.

### 2.3. Analysis of cyclosporin

Whole blood cyclosporin was measured by a specific and selective monoclonal antibody-based radioimmunoassay (RIA) (Sandimmun-kit®, Sandoz Ltd, Basel, Switzerland) which has been shown to be specific for cyclosporin with negligible metabolite cross-reactivity (Ball et al., 1988; Holt et al., 1988). The maximum interassay coefficient of variation was 11.4%.

### 2.4. Pharmacokinetic analysis

A non-linear regression program known as PCNONLIN (Version 4.2; Statistical Consultants, Inc., Lexington, KY, USA) was used to fit individual blood cyclosporin concentrations to a first-order, two-compartment open model. Selection of the most appropriate model was based upon the application of Akaike's criterion (Akaike, 1976). Initial estimates of coefficients and exponentials required by PCNONLIN were obtained from exponential curves by the use of the stripping technique (Gibaldi and Perrier, 1982). Other pharmacokinetic parameters were calculated from the fitted parameters, including the terminal elimination rate constant ($\beta$), terminal elimination half-life ($t_{1/2\beta}$), and area under the blood concentration-time curve (AUC).

Model-independent parameters were also computed. These include the total body clearance of cyclosporin (Cl), steady-state volume of distribution ($Vd_{ss}$) (Benet and Galeazzi, 1979), area under the first moment curve (AUMC), mean residence time of the drug in the body (MRTB) (Yamaoka et al., 1978), mean residence time of the drug in the general circulation (MRTC), mean residence time of the drug in the peripheral tissues (MRTP) and intrinsic mean residence time in peripheral tissue (IMRTP) (Veng-Pedersen, 1989a,b), using the following equations:

$$Cl = dose/AUC$$

$$Vd_{ss} = dose \cdot AUMC/(AUC)^2$$

$$MRTB = AUMC/AUC$$

$$MRTC = AUC/C(0)$$

$$MRTP = MRTB - MRTC$$

$$IMRTP = \frac{MRTP}{1 + \left[C(0)^2/C^1(0) \cdot AUC\right]}$$

where

$$AUMC = \int_0^\infty tC(t)dt$$

$$AUC = \int_0^\infty C(t)dt$$

$$C(0) = A + B$$

and, as defined by Veng-Pedersen (1989a,b),

$$C^1(0) = -(A\alpha + B\beta)$$

where $A$, $\alpha$, $B$ and $\beta$ are hybrid constants of the two-compartment model.

### 2.5. Statistical analysis

The data are presented as mean ± SD. Evaluation was performed in a statistics program (SAS, Statistical Analysis System) using analysis of variance and Student's $t$-test for unpaired data. Differences between two related pharmacokinetic parameters were considered significant for $p$ values less than or equal to 0.05.

## 3. Results

The administration of cyclosporin (15 mg/kg) to the rabbits in the control group and those in the acetazolamide-treated group produced blood concentration-time profiles that were adequately described by the two-compartment model with linear pharmacokinetics. Fig. 1 depicts the time course of the blood cyclosporin level with and without acetazolamide. The calculated compartmental and non-compartmental pharmacokinetic parameters are shown in Table 1. There was no significant difference in the weights of the rabbits

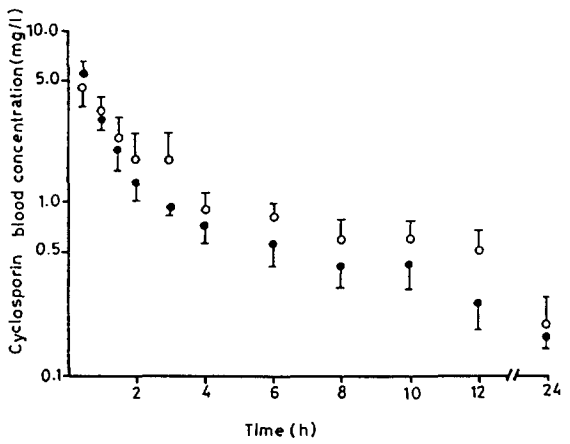


Fig. 1. Mean (±SD) blood concentration of cyclosporin with (○) and without (●) acetazolamide co-administration.

Table 1
Pharmacokinetic parameters of cyclosporin after administration of a single i.v. bolus dose of 15 mg/kg of cyclosporin with and without acetazolamide (i.v., 10 mg/kg) co-administration to rabbits

| Parameter | Cyclosporin alone [a] | Cyclosporin with acetazolamide [a] | $P$ value [b] |
|---|---|---|---|
| AUC ($\mu$g min ml$^{-1}$) | 970.0 ± 108.2 | 1441.3 ± 320.2 | 0.007 |
| Cl (ml min$^{-1}$ kg$^{-1}$) | 15.62 ± 3.78 | 11.0 ± 43.44 | 0.016 |
| Vdss (l kg$^{-1}$) | 4.69 ± 0.95 | 7.57 ± 2.38 | 0.021 |
| MRTB (h) | 5.2 ± 1.4 | 11.7 ± 2.9 | 0.001 |
| MRTC (h) | 1.40 ± 0.2 | 3.6 ± 1.9 | 0.019 |
| MRTP (h) | 3.8 ± 1.3 | 8.1 ± 2.6 | 0.004 |
| IMRTP (h) | 7.4 ± 1.3 | 12.6 ± 3.2 | 0.004 |
| $\beta$ (h$^{-1}$) | 0.125 ± 0.02 | 0.069 ± 0.02 | 0.001 |
| $t_{1/2\beta}$ (h) | 5.7 ± 0.9 | 10.5 ± 2.2 | 0.001 |

[a] Each value represents mean ± SD of six rabbits.
[b] $P$ value of the analysis of variance.

used in the control group (3.82 ± 0.49 kg) and the acetazolamide-treated group (4.07 ± 0.22 kg). The terminal elimination half-life ($t_{1/2\beta}$) of cyclosporin in the acetazolamide-treated group was significantly longer than that in the control group. Compared with the control group, the total body clearance (Cl) of cyclosporin was significantly lower in the acetazolamide-treated rabbits resulting in higher plasma concentrations and a larger area under the blood concentration vs time curve (AUC) in this group. The steady-state volume of distribution of cyclosporin was significantly higher in the acetazolamide-treated rabbits than in the control group. Cyclosporin's mean residence time in the general circulation (MRTC), mean residence time in the body (MRTB), mean residence time in the peripheral tissue (MRTP) and its intrinsic mean residence time in the peripheral tissue (IMRTP) all dramatically increased when administered in conjunction with acetazolamide (Table 1).

## 4. Discussion

Concomitant administration of cyclosporin and acetazolamide was reported to result in increased cyclosporin levels and toxicity (renal impairment and neurotoxicity) in a 50-year-old cardiac transplant patient (Keogh et al., 1988). Symptoms of toxicity subsided following withdrawal of acetazolamide and reduction of the cyclosporin dose.

The results of our investigation demonstrate that co-administration of acetazolamide affected the disposition kinetics of cyclosporin significantly. Acetazolamide administration resulted in a significant decrease in the elimination rate constant (55%), and the total body clearance (29.3%), and a significant increase in the steady-state volume of distribution (61.4%). The reduction in the clearance is confirmed by the significant increase both in the area under the blood concentration-time curve (48.6%), and in the terminal elimination half-life (84%).

Cyclosporin and acetazolamide are known to bind avidly to erythrocyte (Wallace and Riegelman, 1977; Gupta and Benet, 1990; Fahr, 1993). The increase in the steady-state volume of distribution of cyclosporin can be explained, at least in part, by acetazolamide's inhibition of carbonic anhydrase in the red blood cells which may change the way cyclosporin is incorporated into the red blood cells. Another possibility is that acetazolamide displaces cyclosporin from its binding sites. According to Gibaldi and McNamara (1978), the apparent volume of distribution ($Vd_{ss}$) can be related to the free fraction of a drug in blood as follows:

$$Vd_{ss} = V_B + V_T f_B / f_T$$

where $V_B$ is the blood volume, $V_T$ denotes the volume of tissue, $f_B$ is the free fraction in blood, and $f_T$ represents the free fraction in tissue.

The above equation indicates that the apparent volume of distribution at steady state increases when tissue binding increases and blood binding decreases. Perturbation in $f_B$ and/or $f_T$ would therefore alter $Vd_{ss}$.

The decrease in the total body clearance of cyclosporin is difficult to explain. Cyclosporin is mainly eliminated by metabolism (Fahr, 1993), whereas acetazolamide is mainly eliminated unchanged in urine (Bennett et al., 1987). Furthermore, cyclosporin is a low-to-intermediate extraction-ratio drug (Fahr, 1993), and therefore, its total body clearance will depend on the free fraction in blood as well as on its free intrinsic blood clearance according to following equation:

$$Cl = f_B \cdot Cl_{int(blood)}$$

where $Cl_{int(blood)}$ is the intrinsic clearance of unbound drug.

An increase in total body clearance is expected if the free fraction of cyclosporin in blood is increased as a result of altered binding. In contrast, the total body clearance of cyclosporin decreases following acetazolamide administration, suggesting that metabolism inhibition may be responsible. It is possible that acetazolamide co-administration induces metabolic acidosis which results in the reduced hepatic metabolism of cyclosporin, although this warrants further investigation.

The increase in the terminal elimination half-life ($t_{1/2\beta}$) and mean residence time in the body (MRTB) can be explained as follows:

$$t_{1/2\beta} = 0.693 Vd_{ss}/Cl$$

$$MRTB = Vd_{ss}/Cl$$

An increase in $Vd_{ss}$ and a decrease in Cl will result in a significant increase in the $t_{1/2\beta}$ and MRTB.

The calculated mean time parameters which reflect the tissue distribution of cyclosporin are of considerable importance from the toxicokinetic point of view. The mean residence time in the peripheral tissue (MRTP) and the intrinsic mean residence time in the peripheral tissue (IMRTP) both increased dramatically (213 and 70% for MRTP and IMRTP, respectively) with co-administration of acetazolamide. The MRTP is the mean total time the drug molecules spend in the peripheral tissue, while the IMRTP is the average total time which drug molecules spend in the peripheral tissue before being eliminated (centrally or peripherally) from the body (Veng-Pedersen, 1989a,b). It is possible, therefore, to explain the observed toxicity (nephrotoxicity and neurotoxicity) of cyclosporin following acetazolamide administration (Keogh et al., 1988) as being the result of increased distribution into, as well as prolongation of the transit time, in the target organs.

In conclusion, this study demonstrates a possible interaction between acetazolamide and cyclosporin. The most plausible explanation for this is an alteration in disposition kinetics (distribution and elimination) of cyclosporin. Further investigations are needed, however, to identify the exact mechanism of such an interaction.

Cyclosporin and acetazolamide are often given concomitantly in patients with uveitis and secondary glaucoma. This study has shown that the toxicity of cyclosporin may be enhanced by the co-administration of acetazolamide. Consequently, when concomitant use of these drugs is indicated for the treatment of patients with uveitis, a reduction in the dose of cyclosporin and/or acetazolamide may be required to prevent blood cyclosporin levels rising to unacceptable levels.

## Acknowledgements

This study was supported by the Research Center, College of Medicine, King Saud University (Grant No. CMRC-89-263) and the Abdul-Rahman Al-Rashed Fund for Research in Ophthalmology (Grant No. 4-7-92). The authors wish to acknowledge with thanks the technical assistance of Mr M.M. Al-Dardiri and Mr A.A. Abdulraziq.

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