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Pharmacokinetics of cefazoline and dibenzylamine administered in a sustained drug delivery system to healthy volunteers

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Summary

The fate of cefazoline and dibenzylamine following intramuscular injection of a sustained release formulation containing sodium cefazoline : cefazoline-dibenzylamine (1 : 4) at a total dose of 1250 mg was characterized in eight healthy volunteers. Cefazoline and dibenzylamine levels in plasma were determined by HPLC and GLC techniques respectively. Kinetic analysis of the experimental results was performed using a sustained release model for both substances. The maximum plasma levels reached after administration proved to be 50.33 ± 24.33 μg/ml at 1.33 ± 0.52 h for cefazoline and 197.26 ± 136.47 ng/ml at 5.60 ± 3.23 h for dibenzylamine. These findings are a result of the inclusion in the formulation of a fraction of antibiotic subject to immediate release that permits a rapid increase in the plasma levels of cefazoline. The release rate constant of both substances from the derivative has a mean value of 0.095 ± 0.047 h^{-1}. This value is not statistically different ($p > 0.05$) from those of the rate constants governing the disappearance of the antibiotic and dibenzylamine from plasma calculated by the non-compartmental approach. Such findings show that the release of cefazoline and dibenzylamine from the sustained release portion is the rate limiting step in the absorption process and the apparent rate constants calculated from the slope of the terminal phases of the plasma levels curves reflect the value of the release constant of the substances from the derivative. The elimination half-life value of cefazoline calculated from the slope of the terminal phase of the plasma levels curve is significantly higher than that of the conventional formulation. This modification in the elimination half-life allows one to modify the dosage intervals to 24 h, ensuring therapeutic efficiency of treatment.

Introduction

Sustained-release dosage forms are central to the search for improved therapy both through increased patient compliance and a decreased incidence of adverse drug reactions. Over the years, a variety of drug modifications and dosage forms has become available in the attempt to reduce the time course and specificity of drugs in the body (Robinson, 1978). In the field of β-lactam antibiotics, sustained antibacterial activity has been obtained by following the formulation of insoluble derivatives that form a biological depot at the site of administration, with the subsequent slow release to the active form of the drug (Sinkula, 1978).

The formation of this kind of derivatives involves the introduction of a new structure into the molecule that will behave as an independent chemical entity after release of the active compound. Despite the high number of pharmacokinetic studies carried out with this kind of formu-

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lations, such investigations have been limited to examining the kinetic behaviour of the active substance whereas the fate of the other moiety of the derivative is relatively unknown (Lanao et al., 1988).

The aim of the present work was to characterize the fate of cefazoline and dibenzylamine following intramuscular injection of the sparingly soluble salt of cefazoline with dibenzylamine in humans.

Materials and Methods

Drug administration and sampling

The study was undertaken in eight volunteers with normal renal and hepatic functions with an age range between 22 and 30 years (25.2 ± 2.4) and a weight range of 49 and 71 kg (60.2 ± 5.1). The study was approved by the Ethical Committee of the University Clinical Hospital of Salamanca. The volunteers were informed as to the purposes and nature of the trial and gave written consent to participate. They were also subjected to a clinical examination both before starting and after completing the study.

Each of the volunteers received a single intramuscular dose of 1250 mg of the commercial formulation DAREN® composed of a mixture of cefazoline and the dibenzylamine salt of cefazoline at a proportion of 1 : 4.

Venous blood samples (5 ml) were obtained in heparinized tubes at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 9, 12, 24, 36 and 48 h after administation of the formulation. After centrifugation at 2500 g for 15 min, plasma was removed and immediately stored at $-20\,^{\circ}$C until assay.

Sample analysis

Quantification of plasma cefazoline levels was performed by an HPLC technique. 0.1 ml of a deproteinizing solution prepared with 9.5 ml of methanol and 0.5 ml of trichloracetic acid- to which 10 mg of barbital had been added as internal standard- were added to 1 ml of plasma. After shaking the mixture, it was centrifuged at $3000 \times g$ for 3 min. The supernatant fluid was used to determine the antibiotic following injection of 50 μl into the chromatograph.

The apparatus employed was a Varian 5000 chromatograph equipped with a UV detector with a variable wavelength detector at 254 nm. μBondapack C-18 reverse phase columns were employed. The mobile phase was 1/150 M phosphate buffer, pH 7.4, methanol (78 : 22) at a flow rate of 1 ml/min.

Determination of dibenzylamine in plasma was carried out by a gas-liquid chromatographic technique previously published (Calvo et al., 1989). The technique consists in extraction with hexane in basic medium using diphenylamine as internal standard. The column employed was a 3% QF-1 on Gas Chrom a 100/120. The temperatures of the detector (TSD), injector and oven were 250, 210 and 150 °C, respectively. Carrier gas (N_2) flow was 20 ml/min.

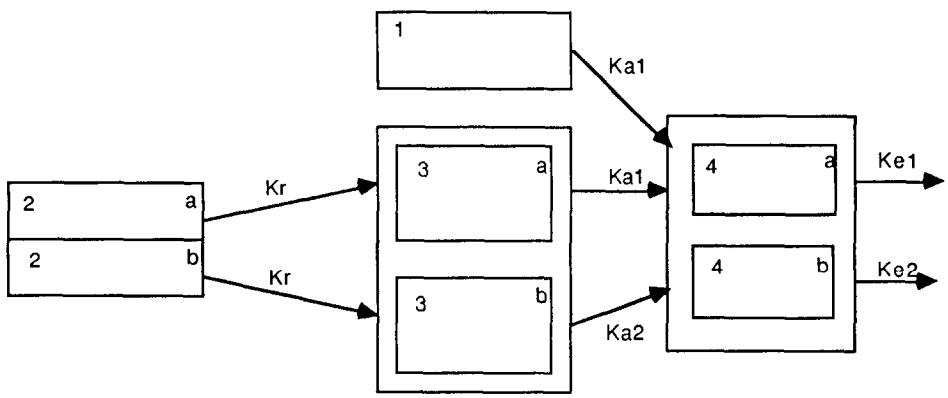

Fig. 1. Pharmacokinetic model proposed for the disposition of cefazoline and dibenzylamine after the administration of the salt.

Pharmacokinetic analysis

Compartmental analysis. The time-course of the plasma levels curves of cefazoline and dibenzylamine obtained after the administration of the formulation were fitted to the model depicted in Fig. 1. In this model, compartment 1 represents the dose of cefazoline instantaneously available for absorption into the systemic circulation according to a kinetic process characterized by its corresponding rate constant, k_{a1}. Compartment 2 represents the derivative to be released slowly at the absorption site. Subcompartments a and b represent the dibenzylamine and cefazoline fractions in the sparingly soluble salt of the antibiotic. The kinetic release process of cefazoline and dibenzylamine from the cefazoline-dibenzylamine salt was characterized by the constant k_r.

The total amount of antibiotic and dibenzylamine available for absorption from the absorption site is represented by compartments 3a and 3b, respectively. The absorption of dibenzylamine is characterized by the first-order constant k_{a2}. The constant defining the absorption of cefazoline released from the cefazoline-dibenzylamine salt is the same as the absorption constant of the instantaneously available cefazoline in the formulation (k_{a1}).

Compartment 4 represents the human body into which the cefazoline and the dibenzylamine are absorbed and eliminated according to a first-order process characterized by their corresponding constants k_{e1} and k_{e2}, respectively. The volume in which both substances are distributed in this compartment is defined by V_1 and V_2, respectively. After defining and solving the system of the differential equations that define the model, it is possible to obtain the following equations to characterize the time-course of cefazoline (C_{cef}) and dibenzylamine (C_{db}) plasma levels, respectively.

$$C_{cef} = \frac{D_{fs}K_{a1}K_r}{V_1(K_{a1}-K_r)(K_{e1}-K_r)}\left(e^{-K_r t}-e^{-K_{e1}t}\right) + \frac{D_{fi}K_{a1}-D_{fs}K_{a1}K_r/(K_{a1}-K_r)}{K_{e1}-K_{a1}} \times \left(e^{-K_{a1}t}-e^{-K_{e1}t}\right) \quad (1)$$

$$C_{db} = \frac{D_{f's}K_{a2}K_r}{V_2}\left[\frac{e^{-K_{a2}t}}{(K_{e2}-K_{a2})(K_r-K_{a2})} + \frac{e^{-K_r t}}{(K_{a2}-K_r)(K_{e2}-K_r)} + \frac{e^{-K_{e2}t}}{(K_r-K_{e2})(K_{a2}-K_{e2})}\right. \quad (2)$$

where $D_{f's}$, D_{fi} and D_{fs} are, respectively, the dose of dibenzylamine, the dose of cefazoline of immediate release, and the dose of cefazoline in the form of the salt with dibenzylamine (Robinson, 1978).

Lanao et al. (1988) have demonstrated that k_a is considerably larger than k_r, such that k_r is the sole rate-determining constant for the absorption of the component of the insoluble derivative; thus eqns 1 and 2 can be approximated by:

$$C_{cef} = \frac{D_{fs}K_r}{V_1(K-K_{e1})}\left(e^{-K_{e1}t}-e^{-K_r t}\right) + \frac{D_{fi}K_{a1}}{V(K_{a1}-K_{e1})}\left(e^{-K_{e1}t}-e^{-K_{a1}t}\right) \quad (3)$$

$$C_{db} = \frac{D_{f's}K_r}{V_2(K_r-K_{e2})}\left(e^{-K_{e2}t}-e^{-K_r t}\right) \quad (4)$$

The fitting of the experimental results to the proposed pharmacokinetic model was carried out using a non-linear regression program based on the Nelder and Mead algorithm (Statistical Consultants, 1986). The weighting factor used was the reciprocal of the value of the plasma concentration of the cefazoline and dibenzylamine. Validation of the proposed pharmacokinetic model was peformed by analysis of the residuals and standard deviations of the parameters obtained by non-linear regression.

Non-compartmental analysis. The rate constant of the descending phase of the plasma levels of cefazoline and dibenzylamine (k_1 and k_2) was calculated from the terminal phase of the plasma levels curves of both substances. The constant governing the ascending phase of the plasma levels curve (k_{a1} and k_{a2}) was calculated by the strip-

ping procedure. The area under the curve of the plasma concentrations (AUC) from time zero to infinity was calculated from the area under the curve from time zero up to 48 h (obtained by the linear trapezoid method) plus C_{48} h/K, where C_{48}h is the plasma concentration value at 48 h after administration, and K is the rate constant of the terminal phase of the plasma levels (Gibaldi and Perrier, 1982).

The mean residence time of cefazoline and dibenzylamine was calculated by the following equation:

$$\text{MRT} = \frac{\int_0^\infty tC_p\,dt}{\int_0^\infty C_p\,dt} = \frac{\text{AUMC}}{\text{AUC}} \qquad (5)$$

where AUMC and AUC are the first moment of the area under the plasma levels curve and the area under the curve of the plasma levels, respectively (Yamaoka et al., 1978).

The maximum plasma concentration (C_{max}) and the time taken to reach this concentration (t_{max}) were determined from the curves of cefazoline and dibenzylamine, respectively.

Non-compartmental analysis was performed using the pKcalc program on an IBM-AT computer (Shumaker, 1986).

Statistical analysis. In order to compare the values of the different parameters obtained by compartmental and noncompartmental analysis, and with those of previous studies, a one-way ANOVA variance test was performed (Rosner, 1982).

Results and Discussion

Fig. 2 shows the time course of the plasma levels of cefazoline and dibenzylamine after i.m. administration of 1250 mg of the formulation. It can be seen that there is good agreement between the experimental and theoretical values for both

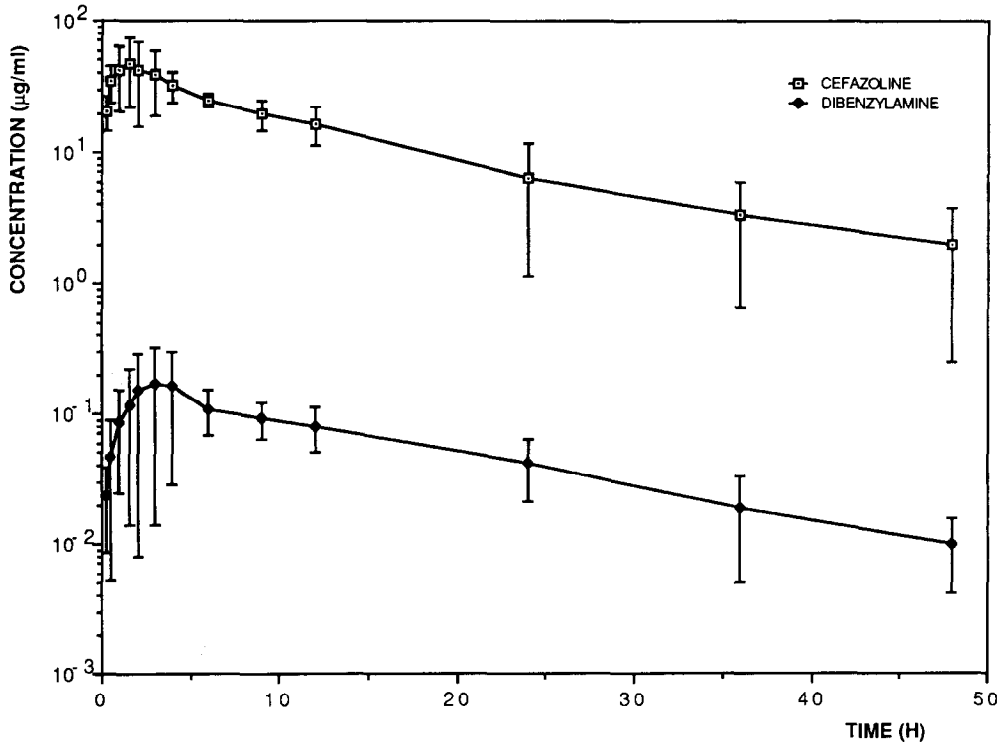

Fig. 2. Plasma levels of cefazoline and dibenzylamine following i.m. administration of 1250 mg of Daren® to healthy volunteers.

TABLE 1

Pharmacokinetic parameters of cefazoline and dibenzylamine obtained with compartmental analysis

	K_r (h^{-1})	K_{a1} (h^{-1})	K_{e1} (h^{-1})	K_{e2} (h^{-1})	V_1 (l)	V_2 (l)
$\bar{X}$	0.095	3.05	0.406	0.360	0.705	6.81
σ_{n-1}	0.047	1.83	0.288	0.095	0.580	2.08

substances obtained according to the model offered in Fig. 1.

Tables 1 and 3 show the pharmacokinetic parameters obtained using the compartmental and non-compartmental approaches, respectively.

The maximum plasma levels reached proved to be 50.33 ± 24.33 μg/ml and 197.26 ± 136.47 ng/ml for cefazoline and dibenzylamine, respectively. The time at which these maximum values were reached was significantly higher in the case of dibenzylamine than for cefazoline. These findings are a result of the inclusion in the formulation of a fraction of antibiotic subject to immediate release that permits a rapid increase in the plasma levels of cefazoline.

Table 1 shows that the value of the release constant of cefazoline and dibenzylamine from the derivative has a mean value of 0.095 ± 0.047 h^{-1}. On comparing this value with those of the rate constants governing the disappearance of the antibiotic and dibenzylamine from plasma calculated by the non-compartmental approach (0.083 ± 0.048 and 0.094 ± 0.057 h^{-1}, respectively), it is seen that there are no statistically significant differences ($p > 0.05$) between them. Likewise, these values are not very different from those reported by Lanao et al (1988) using the Wagner-Nelson method (0.068 ± 0.017 h^{-1}) and statistical moments (0.094 ± 0.035 h^{-1}) for the same parameter. Such findings show that the release of cefazoline and dibenzylamine from the sustained release portion is the rate limiting step in the absorption process and the apparent rate constants calculated from the slope of the terminal phases of the plasma levels curves of cefazoline and dibenzylamine reflect the value of their release constant from the insoluble salt.

The absorption constant of cefazoline (k_{a1}) has a mean value of 3.05 ± 1.83 h^{-1} this is similar to the value obtained for the absorption constant of cefazoline when administered intramuscularly in the conventional formulation. In both cases (conventional formulation and sustained action form), this parameter displays considerable interindividual variability; greater than 50%.

The elimination constant of cefazoline obtained in the present study was 0.360 ± 0.095 h^{-1}. This value was also very similar to the elimination constant of cefazoline when administered as the conventional formulation (0.332 ± 0.098 h^{-1}).

These results allow us to conclude that parameters calculated in our study and shown in Table 1 are not apparent parameters, but rather represent their intrinsic values, thus allowing one to justify the validity of the proposed pharmacokinetic model. The mean residence times of both substances were 14.61 ± 5.34 and 15.50 ± 5.68 h for cefazoline and dibenzylamine, respectively; these values are very similar, with no statistically significant differences between them. The area under the

TABLE 2

Pharmacokinetic parameters of cefazoline obtained with non-compartmental analysis

	K_{a1} (h^{-1})	C_{max} (μg/ml)	t_{max} (h)	K_{e1} (h^{-1})	$t_{1/2}$ (h)	AUC (μg h ml^{-1})	MRT (h)
$\bar{X}$	5.59	50.33	1.33	0.083	10.05	532.26	14.61
σ_{n-1}	4.40	24.33	0.52	0.048	3.69	116.81	5.34

TABLE 3

Pharmacokinetic parameters of dibenzylamine obtained with non-compartmental analysis

	K_{a2} (h^{-1})	C_{max} (ng/ml)	t_{max} (h)	K_{e2} (h^{-1})	$t_{1/2}$ (h)	AUC (ng h ml^{-1})	MRT (h)
$\bar{X}$	0.629	197.26	5.60	0.094	8.76	2511.21	15.51
σ_{n-1}	0.560	136.47	3.23	0.057	3.04	395.85	5.68

curve of the plasma levels of cefazoline and dibenzylamine lay around 500 μg h ml^{-1} and 2500 ng h ml^{-1}, respectively.

The elimination half-life values of cefazoline and dibenzylamine calculated from the slopes of the terminal phase of the plasma levels curves were 10.05 ± 3.69 and 8.76 ± 3.04 h, respectively; these are significantly different from that of the elimination half-life of the conventional formulation (2.22 ± 0.52 h) (Lanao et al., 1988). This modification in the elimination half-life allows one to introduce important changes in the dosage intervals of the antibiotic that can be administered at intervals of 24 h, ensuring therapeutic efficiency of treatment. Additionally, the accumulation of the other component of the sustained action formulation, dibenzylamine, is practically nonexistent; thus, the appearance of possible side effects due to this substance is avoided.

References

Calvo, M.B., Pedraz, J.L. and Domínguez-Gil, A., Determination of low concentrations of dibenzylamine in human plasma and urine by gas chromatography with a nitrogen-phosphorus detector. *J. Chromatogr. Biomed. App.*, 490 (1989) 206–212.

Gibaldi, M. and Perrier, D., *Pharmacokinetics*, 2nd Edn, Dekker, New York, 1982.

Lanao, J.M., Vicente, M.T. and Domínguez-Gil, A., Pharmacokinetics of cefazolin administered as a new drug delivery system in healthy volunteers. *Biopharm. Drug Dispos.*, 9 (1988) 377–388.

Robinson, R., *Sustained and Controlled Release Drug Delivery Systems*, Dekker, New York, 1978.

Rosner, B., *Fundamentals of Biostatistics*, Duxbury, Boston, 1982.

Shumaker, R.C., PKCALC: A BASIC interactive computer program for statistical and pharmacokinetic analysis of data. *Drug Metab. Rev.* 17 (1986) 331–348.

Sinkula, A.A., Methods to achieve sustained drug delivery. In Robinson, R. (Ed.), *Sustained and Controlled Release Drug Delivery Systems*, Dekker, New York, 1978, pp. 466–468.

Statistical Consultants, Inc. *Am. Statistician*, 40 (1986) 52–55.

Yamaoka, K., Nakagawa, T. and Uno, T., Statistical moments in pharmacokinetics. *J. Pharmacokinet. Biopharm.*, 6 (1978) 547–558.