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Oral administration of liposomes containing cyclosporine: a pharmacokinetic study

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Abstract

Liposomal formulation containing cyclosporine A (CSA) were prepared. The most stable liposomes with the composition of CSA, dipalmitoylphosphatidyl choline (DPPC) and cholesterol (Chol.) in molar ratio 1:0.2:1, respectively were administered orally to New Zealand rabbits. The pharmacokinetic of the administered CSA was compared with that of the commercially available oily oral formulation of CSA (Sandimmune) at dose of 15 mg/kg. Cyclosporine concentration in blood was monitored using a radioimmunoassay method (RIA). A change in the pharmacokinetic parameters of CSA due to liposomal encapsulation was observed. A peak concentration was reached in 50 min in case of liposomes compared with 225 min in case of Sandimmune. The rate of absorption ($C_{max}/AUC_{0-\infty}$) was significantly faster following the liposome administration. A significant difference in the area under the concentration curve ($AUC_{0-\infty}$) was found and this was attributed to the difference in the terminal half-lives ($t_{1/2}\beta$) which were 8.88 ± 1.94 and 19.3 ± 8.48 h for liposomes and Sandimmune preparations, respectively. The mean residence time (MRT) and the mean absorption time (MAT) were dramatically decreased following the administration of liposomal formulation. Generally, there was less inter-individual variation in the values of rate of absorption, $t_{1/2}\beta$ and MRT when CSA liposomes were orally administered compared to the administration of Sandimmune. Thus, an oral liposomal formulation for CSA can be developed to offer the advantages of low variability and fast onset of action.

Keywords: Cyclosporine; Liposomal formulation; Oral administration; Pharmacokinetics

1. Introduction

Cyclosporine A (CSA) is considered as one of the most effective immunosuppressive drugs used

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today for the prevention of allograft rejection after organ transplantation (Kahan, 1989). In spite of the great medical importance of the drug, some currently available dosage forms suffer several disadvantages. These include slow and high variability of CSA absorption following the oral administration of its oily solution (Ptachcinski et al., 1986). The polyoxyethylated castor oil (Cremophor EL) used to dissolve CSA in the intravenous (I.V.) formulation was reported to cause anaphylactic shock (Cavanak and Sucker, 1986) and to induce nephrotoxic effects similar to those produced by CSA (Luke et al., 1987). Due to the low solubility and the difficulty of solubilizing CSA in aqueous vehicles (Khidr, 1987), no safe commercial substitute to Cremophor has been yet identified. Several alternative dosage forms have been proposed to overcome these problems. Among these are a liposomal formulation for I.V. administration (Venkataram et al., 1990; Vadiei et al., 1989), and for aerosol delivery direct to the pulmonary tissue (Waldrep et al., 1993). A controlled release parenteral delivery system for CSA in biodegradable microspheres and nanospheres was also suggested (Sanchez et al., 1993). A lipophilic carrier for oral CSA administration was tried, but proved to have limited in vitro and in vivo stability (Yanagawa et al., 1989). Recently, a new oral formulation (Sandimmune Neoral) was developed which incorporated the drug in a microemulsion preconcentrate containing a surfactant, lipophilic and hydrophilic solvents, and ethanol (Muller et al., 1994). The greater toxicity from the currently I.V. CSA formulation than from the oral dosage form (Williams et al., 1986) and the need to have a rapid onset of the immunosuppressive effects in transplanted patients necessitate the search for an oral dosage form that might reduce the toxic effects and improve pharmacokinetics of CSA.

Although liposomes are expected to be unstable in the G.I.T., they have been found to improve the systemic absorption of labile compounds after oral administration (Fielding, 1991) and they may act as a non-toxic vehicle for insoluble drugs (Lidgate et al., 1988). In addition they can alter tissue distribution of drugs within the body. Thus, liposomes containing cyclosporine may help reduce the nephrotoxicity of CSA (Venkataram et al., 1990).

A number of liposomal CSA formulations have previously been developed and evaluated in our laboratory (Al-Angary et al., 1995). This included multilamellar vesicles composed of DPPC with and without cholesterol (Chol.) at different molar ratios. The purpose of the present work was to study the pharmacokinetics of CSA formulated in the most promising stable liposomal batch after oral administration to rabbits and to compare it to the commercially available oral Sandimmune® oily solution.

2. Materials and methods

2.1. Materials

L-α-Dipalmitoylphosphatidyl choline (DPPC) and cholesterol (Chol.) were purchased from Sigma (St. Louis, MO, USA). Cyclosporine A (CSA) and cyclosporine D (CSD) were gifts from Sandoz Pharma, Switzerland. Commercially available Sandimmune® I.V. and oral formulations were purchased from the local market. Other solvents and materials were of analytical grade.

2.2. Preparation and in vitro evaluation of CSA liposomes

Multilamellar vesicles (MLV's) were prepared following the film method (Bangham et al., 1965). The method of preparation as well as the in vitro evaluation of produced liposomes were described with details in a previous study (Al-Angary et al., 1995). The liposomal formula chosen for in vivo study consisted of DPPC, CSA and Chol. at molar ratios 1:0.2:1 respectively, as it was the most stable. The particle size of the prepared liposomes was 4.34 ± 1.43 μm as determined using a photomicroscope (Nikon Model UFX-II, Japan) at 1000X magnification.

2.3. Animal study

Fourteen New-Zealand white male rabbits, weighing between 3.0–4.0 kg, with an average

weight of 3.55 ± 0.54 kg were used in this study. The rabbits were fasted overnight but were allowed free access to water. Each animal received 15 mg/kg of CSA dose in one of the following dosage forms: (1) CSA commercial oral Sandimmune ($n = 5$); (2) CSA commercial I.V. ($n = 4$); and (3) CSA liposomal formulation for oral administration that was prepared in our laboratory ($n = 5$). The oral doses were administered using polyethylene tube while the marginal ear vein was used for the I.V. dosing with the aid of implanted cannula for collecting blood samples. The samples (≈ 1.5 ml) were collected in tubes with EDTA prior to and at intervals up to 36.0 h post oral administration and prior to and up to 5.0 h post I.V. administration. The samples were stored at -20°C pending analysis.

2.4. Drug analysis

Cyclosporine concentrations in blood samples were analyzed using a radioimmunoassay method (Cyclo. Trac. SP. I-125 RIA kit, Drug International, NJ, USA). The assay was performed according to the manufacturer's instructions. The samples were combined with the iodine-125 cyclosporine tracer and antibody reagent. Following a 1 h incubation at 20–25°C, the tubes were centrifuged, decanted, and then counted using a γ scintillation counter (Mini γ 1275, KLB, Turku, Finland). A standard curve was constructed between 70 and 1500 ng/ml. The mean sensitivity for the assay was 8.7 ng/ml. Within-batch coefficient of variation (CV) values were less than 5%, while, between-day CV values were within 10% at different concentrations. The average recovery for CSA using this method was 95.1%.

2.5. Pharmacokinetic analysis

Pharmacokinetic parameters for CSA following oral administration of commercial Sandimmune and the liposomal preparations were determined from the concentration-time data. The maximum blood concentration (C_{max}) and the time to reach this maximum (T_{max}) were obtained directly from the individual concentration- time profiles. The apparent elimination rate constant (K_{el}) was estimated by the least-square regression analysis of the final segment of the curve and the terminal elimination half-life ($t_{1/2}\beta$) was calculated as $0.693/K_{el}$. The area under the concentration-time curve (AUC) and the area under the moment curve (AUMC) were estimated by the linear trapezoidal rule and extrapolated to infinity using standard methods. The mean residence time (MRT) was calculated as the ratio of $AUMC_{0-\infty}$ to $AUC_{0-\infty}$ and the mean absorption time (MAT) as the difference between MRT_{p0} and $MRT_{I.V.}$, where MRT_{p0} is the mean residence time after oral administration and $MRT_{I.V.}$ is the mean residence time after I.V. administration. The rate of absorption was also calculated using the equation: $C_{max}/AUC_{0-\infty}$.

2.6. Statistical analysis

The pharmacokinetic data of CSA following oral administration were compared statistically using analysis of variance (ANOVA) at a significant level ($p \leq 0.05$). Variation between animals in the pharmacokinetic parameters following oral administration of each dosage form were expressed by the coefficient of variation (CV, %).

3. Results and discussion

Based on the in vitro evaluation and the in vivo targeting studies (Al-Angary et al., 1995), a liposomal formulation containing a high Chol-level was chosen for the bioavailability studies. Fig. 1 shows the mean blood CSA concentration versus time profiles after oral administration of CSA in two different dosage forms at 15 mg/kg to rabbits; namely: commercially available oily solution (Sandimmune®) and the selected liposomal formulation. The mean pharmacokinetic parameters of CSA following oral administration of the two products are shown in Table 1.

A peak concentration was reached in less than 50 min in case of the liposomes while it took 225 min in the case of Sandimmune, indicating faster absorption of CSA from liposomes than from the commercially available oily solution. Although there was no significant difference in the maxi-

Table 1
Pharmacokinetic parameters of CSA following administration of the two oral preparations (15 mg/kg) to rabbits (Mean ± S.D.)

Preparations	Parameters						
	C_{max} (μg/l)	T_{max} (h)	$AUC_{0-\infty}$ (μg·h/l)	$C_{max}/AUC_{0-\infty}$ (h^{-1})	$t_{1/2}\beta$ (h)	MRT (h)	MAT (h)
Liposomes	927 ± 208 (22.4)[b]	0.8 ± [a]0.27 (33.7)[b]	11177.0[a] ± 3637 (32.5)[b]	0.085[a] ± 0.013 (15.3)[b]	8.88[a] ± 1.94 (21.8)[b]	12.4[a] ± 2.67 (21.5)[b]	8.24[a]
Sandimmune®	1166 ± 146.3 (12.6)[b]	4.0 ± 0.60 (15.0)[b]	19171.7 ± 4611 (24.0)[b]	0.063 ± 0.014 (22.2)[b])	19.13 ± 8.48 (44.3)[b]	23.2 ± 6.74 (29.9)[b]	19.06

[a] $p < 0.05$ from Sandimmune® commercial preparation.
[b]CV, % values between parenthesis.

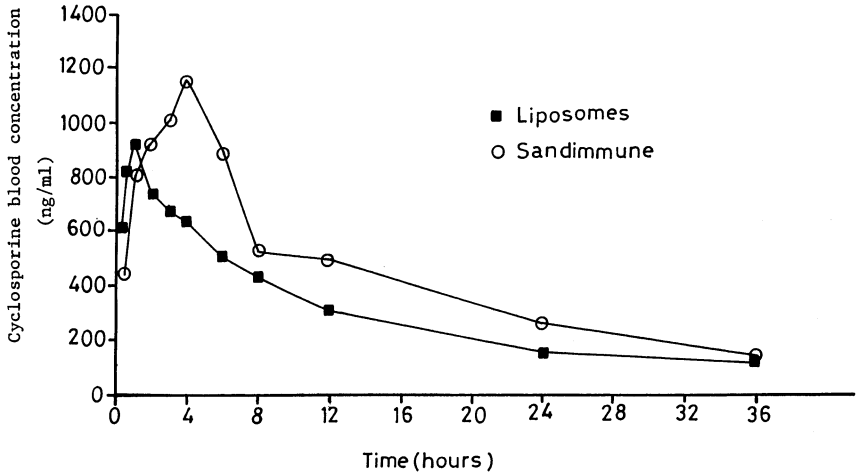

Fig. 1. Mean blood concentration–time profile of CSA (15 mg/kg) following the oral administration of Sandimmune and liposomes to rabbits.

mum concentration (C_{max}) between the two dosage forms ($p > 0.05$), a statistically significant difference in the absorption rate defined as $C_{max}/AUC_{0-\infty}$ was found. The rapid rate of CSA absorption from the liposomes may be attributed to the fast release of the drug from the liposomal vesicles in the gastrointestinal tract and–or the fast absorption of drug entrapped in the phospholipid carrier. Following the oral administration of Sandimmune, the rate of CSA absorption is determined by the dispersion of the oil droplets in the aqueous gastrointestinal fluid (Reymond et al., 1988) which came to be slower and showed higher individual variability compared with liposomes.

Table 1 shows also that the half-life of CSA from the orally administered liposomal formulations was nearly half that of the Sandimmune oral preparation. The variability, in terms of the standard deviation, from the mean was much less in case of the liposomal formulation compared with Sandimmune. Knowing that the pharmacokinetic parameters of CSA oral dosage forms now available show large inter- and intra-individual variations (Ptachcinski et al., 1986; Luke et al., 1992), our liposomal preparation is perhaps more favourable and advantageous oral dosage form for CSA in this respect.

The results also show that the area under the concentration-time curve from time zero to infinity ($AUC_{0-\infty}$) is significantly greater in case of Sandimmune than the liposomal formulation ($p < 0.05$). The difference in the extent of absorption may be attributed mainly to the apparent differences in the half-life. The relative bioavailability of the liposomal formulation to that of the commercial oral preparation found to be about 60%.

The mean residence time after i.v., $MRT_{i.v.}$, and after the oral administration of the liposomal formulation, MRT_{lip}, and commercial product MRT_{Com}, to rabbits were found to be 4.16 ± 0.47, 12.4 ± 2.67, and 23.22 ± 6.74 h, respectively. The mean absorption time (MAT), calculated by subtracting $MRT_{i.v.}$ from MRT_{lip} and MRT_{Com}, was found to be 8.24 and 19.06 h, respectively. The MAT values of Sandimmune is 2.31 times greater than that of oral liposomes which clearly demonstrate a longer time period for absorption of CSA from Sandimmune. It was reported that altered absorption of cyclosporine is accompanied by a change in $t_{1/2}$ (Muller et al., 1994). In comparing the values of $t_{1/2}\beta$ for the three administered dosage forms it was noticed that there is an increase in the apparent $t_{1/2}\beta$ of CSA after administration of oral Sandimmune with the value of 19.13 h compared with 8.88 h for oral liposomes and 8.47 h for I.V. administration of the commercial product. This is probably due to slow release

of CSA from oily solution and hence slow absorption in the presence of fast elimination, a case represented by a 'Flip-Flop' pharmacokinetic model (Gibaldi and Perrier, 1982; Avgerinos and Gorrod, 1990). The slower absorption from oily solution is also supported by the prolonged T_{max}. Furthermore, the calculated rate of absorption (C_{max}/AUC) was in favour of liposomes (Table 1). Generally, the rate and extent of CSA absorption came to be different for the two formulations and this was manifested by longer T_{max} and higher AUC value for the oily solution.

The CV% values for $C_{max}/AUC_{0-\infty}$, MRT, and $t_{1/2}\beta$ (Table 1) were markedly lower in case of liposomal administration compared with oral Sandimmune. This may indicate less inter-individual variation in the rate of absorption in case of liposomal administration.

Based on this work, it is believed that an alternative liposomal formulation for oral administration can be obtained from which the CSA can exert its clinical effects with minimum interindividual variations.

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