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Determination of serum diltiazem concentrations in a pharmacokinetic study using gas chromatography with electron capture detection*

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Abstract: An open cross-over randomized clinical trial was performed in nine healthy humans to determine steady-state pharmacokinetics and bioavailability of three oral diltiazem preparations, tablets containing 60 and 90 mg of diltiazem hydrochloride, administered in total daily doses of 180 mg. Serum drug levels were determined by gas chromatography with electron capture detection following a simple extraction procedure. Blood samples were collected before and at several post-dosing intervals after administration of the last dose in steady state, and pharmacokinetic parameters were calculated. The steady-state diltiazem concentrations in sera were determined 48 h after the first dose, and were (mean ±SD): 46.4 ± 28.1, 60.8 ± 36.3 and 36.8 ± 22.6 μg l^{-1} for Pliva 60, Pliva 90, and Aldizem 90 diltiazem preparations, respectively. The corresponding elimination half-lives were 5.6 ± 2.0, 5.2 ± 1.8 and 6.9 ± 3.2 h; peak concentrations were 88.4 ± 29.5, 153.5 ± 86.5 and 139.2 ± 72.5 μg l^{-1}, and areas under the concentration curves (AUC 12 h) were 477.4 ± 172.5, 989.2 ± 536.3 and 817.9 ± 494.5 μg h l^{-1}, respectively.

Keywords: *Diltiazem; steady-state pharmacokinetics; GC–ECD.*

Introduction

Diltiazem, D-*cis*-(+)-3-(acetyloxy)-5-[2-(dimethylamino)-ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-1,5-benzothiazepine-4-(5H)-one is a calcium channel antagonist mainly used in the treatment of various types of cardiovascular disease [1, 2]. Studies in patients suffering from coronary spasm have revealed correlation with the dose administered [3], while results obtained by oral administration of diltiazem tablets containing different doses highlight the importance of a proper choice of dose size and administration frequency for maintenance of drug concentration in blood within a desired range [4]. Although a review of pharmacological properties and therapeutic efficacy of diltiazem is available [5], the difficulties encountered when analysing this drug in biological matrix (to determine the blood therapeutic levels), as well as its behaviour in long-term treatment, is a topic of several analytical and pharmacokinetic studies. The pharmacokinetic behaviour of diltiazem was studied by Koelle and coworkers [6] following i.v. and p.o. administration of several diltiazem doses in six healthy subjects. It was characterized by a three-compartment open model describing a rapid initial disposition phase and a slow terminal elimination phase. Following tablet administration, C_{max} were found after 2.8 ± 0.9 h. Although only a minor part of diltiazem is eliminated during the terminal gamma-phase, the authors stress that it should give rise to a certain extent of drug accumulations at therapeutic dosage regimens [6].

Diltiazem and six of its metabolites in plasma were studied in four healthy volunteers administered a single 90-mg oral dose [7]. The peak diltiazem concentrations occurred 3–4 h post-administration, ranging from 86 to 188 μg l^{-1}, and the elimination half-lives ranged from 3.5 to 5.3 h. The pharmacokinetics of different dose (30–120 mg) sustained release tablets was also studied in healthy American men [8]. The authors report wide variability among individuals after administration of a single

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dose. They also noticed that the AUCs also tend to increase more than the multiple of the dose administered.

Steady-state pharmacokinetics of diltiazem was recently published by Hoeglund and Nilsson [9]. They compared two orally multiple dose regimens (60 mg t.i.d. and 120 mg t.i.d.) during 14 days treatment. A two-compartment model was chosen to describe the disposition of diltiazem. The elimination was found to be biphasic in all individuals, with $t_{1/2}$ of 1.54 ± 0.55 and 1.46 ± 0.61 h in the first elimination phase, and 6.57 ± 1.41 and 5.44 ± 0.66 h in the terminal elimination phase after t.i.d. treatments with 60 and 120 mg, respectively. The mean concentrations in the steady state (C_{ss}) were after 60 mg t.i.d. 214 ± 116 nmol l^{-1} diltiazem (corresponding to 96.5 ± 52.3 μg l^{-1}), and after 120 mg t.i.d. 475 ± 199 nmol l^{-1} (corresponding to 214.2 ± 89.7 μg l^{-1}) diltiazem.

Steady-state pharmacokinetics of diltiazem in healthy subjects after once-daily administration of 90, 120, 180 and 240 mg retard capsules was recently published [10].

Therefore, we decided to determine the bioavailability and pharmacokinetics of three oral diltiazem preparations in the steady state. Tablets containing 60 mg (Pliva 60) and 90 mg (Pliva 90) of diltiazem hydrochloride were compared with tablets containing 90 mg of the same active substance formulated by another manufacturer (Aldizem 90; Alkaloid — Skopje, Yugoslavia); the clinical trial was performed in steady-state conditions.

Materials and Methods

An open cross-over randomized clinical trial was performed in nine healthy human volunteers to determine pharmacokinetics and bioavailability of three oral diltiazem preparations. The trial protocol was approved and accepted by the Hospital Ethical Drug Committee.

Subjects

Nine healthy volunteers of both sexes, students aged 21–36 years (mean ±SD = 24.2 ± 4.5), body weight 54–88 kg (mean ±SD = 75.2 ± 13.4), were medically examined and informed in detail of the plan and aim of the trial as well as of the possible side effects. They were obliged to sustain from any drugs or alcohol for at least 7 days before the beginning and during the trial.

Experiment

The interval between the experimental series was 7 days. Each diltiazem preparation was administered in a total daily dose of 180 mg (divided into three 60-mg doses administered at 8 a.m., 2 p.m. and 10 p.m., or two 90-mg doses administered at 8 a.m. and 8 p.m.) for 2 days (48 h) until the steady-state concentration was reached. On the third day, only the morning dose was administered, and blood samples were collected before, and 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8 and 12 h post-dose. Serum samples were separated by centrifugation and stored at −20°C until analysis.

Diltiazem preparations

Tablets containing 60 and 90 mg of diltiazem hydrochloride (Pliva 60 and Pliva 90, respectively) were manufactured by Pliva, Zagreb, whilst Aldizem 90 mg tablets were manufactured by Alkaloid (Skopje, Yugoslavia).

Materials

All chemicals used were of p.a. quality. Deionized water was tested for gas chromatography with electron capture detection (GC–ECD) suitability, while methanol and *n*-hexane were distilled before use.

Analytical method

To 1 ml of serum in a glass-stoppered test-tube, 1 ml of 0.067 mol l^{-1} phosphate buffer (pH 7.8) containing internal standard (flurazepam monohydrochloride 20 ng) was added. After short vortex-mixing, 5 ml of *n*-hexane was added and diltiazem extracted into organic solvent by gently rotating the glass-stoppered test-tubes for 15 min. The organic layer was separated by centrifugation (5 min at 2500*g*), transferred to another test-tube and evaporated to dryness in a stream of nitrogen at 40°C. The residue was redissolved in 0.1 ml of methanol and analysed by GC–ECD. All the steps from blood collection to GC were performed in the dark to minimize degradation of diltiazem.

The chromatographic system consisted of Shimadzu GC-9A gas chromatograph with 63Ni electron capture detector and Shimadzu Chromatopac C-R3A integrator. The column used (glass, 2 m × 3 mm, i.d.) was packed with 1% OV-17 on a Chromosorb W (AW-DMCS),

100–120 mesh, and operated at 280°C, while the injector and detector temperatures were set to 300°C, and nitrogen flow to 50 ml min^{-1}. Under the above conditions, internal standard (flurazepam) and diltiazem retention times were 2.20 and 4.24 min, respectively. The peak area ratio of diltiazem and internal standard was linearly related to the amount of diltiazem added to blank plasma.

Calculations

Statistical comparisons were made using the ANOVA test. The AUCs were calculated using the trapezoidal method.

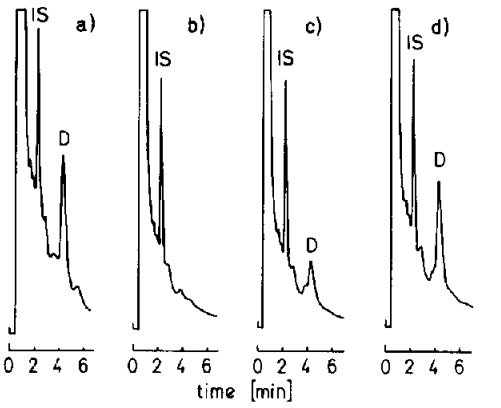

Figure 1
Chromatograms of the same single volunteers serum containing internal standard (flurazepam; IS, 20 ng ml^{-1}) and processed by the method described: (a) blank serum spiked with diltiazem (D) 100 ng ml^{-1}, (b) blank serum taken before the first dose of diltiazem, (c) serum in steady-state condition containing diltiazem (D) 30 ng ml^{-1}, (d) serum sample taken 2.5 h after the last 90 mg dose of diltiazem (D, 86.8 ng ml^{-1}).

Results and Discussion

The analytical method presented here is based on some previously published papers [11, 12] with certain modifications that made it simpler and more suitable for bioavailability studies. The throughput of the extraction step was within the range of about 60 serum samples per day, whilst the gas chromatography allowed about six samples per hour. The assay quantification limit was 5 ng diltiazem per ml serum (LOQ), although 2 ng ml^{-1} could also be detected (LOD). The precision was 3% at 100 ng ml^{-1} serum (n = 9) and the extraction efficiency averaged 95%. Linearity was retained up to 500 ng ml^{-1} serum. No interference was observed from either lipemic or haemolysed blood samples. Figure 1 shows the typical chromatograms. This method was used to determine diltiazem in sera of nine healthy humans. They volunteered in a bioequivalence study comparing diltiazem tablets from two manufacturers. As the therapeutic efficiency of diltiazem depends on the steady state in blood [3], the tested preparations were administered in a daily dose of 180 mg until the steady-state concentration was reached (Table 1). Diltiazem concentrations determined in serum collected before the last dose and at several post-dosing intervals are shown in Fig. 2. The pharmacokinetic parameters (Table 1) were calculated from the measured diltiazem concentrations (n = 9) for each examined preparation. It can be noticed that the residual diltiazem concentrations (Table 1, C_{SS} differed by 40% in the two 90 mg diltiazem preparations (manufactured by two different producers), which stresses the importance of the bioavailability and bioequivalence testing for all new or parallel drug formulations. The difference in relative bioavailability (F) between the 60 mg (Pliva 60) and 90 mg (Aldizem 90) doses was 12% after correction on the basis of dose diltiazem content, and was not significantly different.

Table 1
Pharmacokinetic parameters obtained from the mean diltiazem concentrations in healthy humans (n = 9)

Pharmacokinetic parameter	Diltiazem preparations Pliva 60 Mean	SD	Pliva 90 Mean	SD	Aldizem 90 Mean	SD
C_{ss} (μg l^{-1})	46.4	28.1	60.8*	36.3	36.8*	22.6
AUC_{12h} (μg h l^{-1})	477.4* **	172.5	989.2*	536.3	817.9**	494.5
$t_{½el}$ (h)	5.6	2.0	5.2	1.8	6.9	3.2
C_{max} (μg l^{-1})	88.4**	29.5	153.5**	86.5	139.2**	72.5
T_{max} (h)	2.11**	0.74	2.33**	0.71	2.89	0.99
$F_{corrected}$ (%)	87.5		121		100	

*$p < 0.02$.
**$p < 0.05$.
F (relative bioavailability) was corrected on the basis of dose content of diltiazem for Pliva 60 mg preparation.

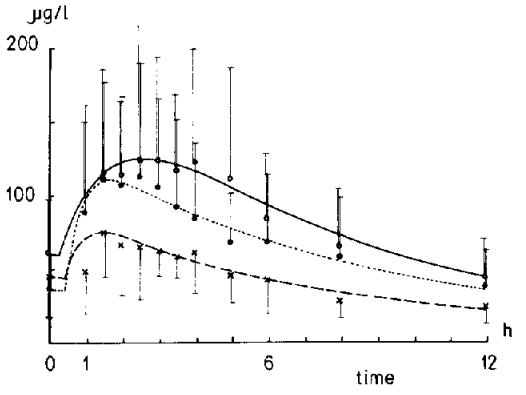

Figure 2
Mean diltiazem serum levels (mean ± SD) after administration of the last dose of the examined preparation in steady-state conditions (n = 9): ---x--- Pilva 60 mg, —○— Pilva 90 mg, ····●···· Aldizem 90 mg; and the simulated concentration curves for each of the preparations.

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