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Short communication

A selective HPLC method for the determination of indapamide in human whole blood: Application to a bioequivalence study in Chinese volunteers

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Abstract

Indapamide was extracted from human whole blood with diethyl ether and was determined by a HPLC-UV method using an Inertsil ODS-3 column and an isocratic mobile phase consisting of 55% buffer solution (2 g KH_2PO_4, 3 ml H_3PO_4 and 3.5 ml triethylamine in 1 l of H_2O), 40% acetonitrile and 5% methanol for 12.5 min, and then a gradient flush from 100% isocratic to a mixture of 20% isocratic mobile phase and 80% methanol for 3 min. Indapamide and glipizide (internal standard) were eluted from the column at about 10.5 and 12.8 min, respectively. The method had within day precision values in the range ±1.2 to ±9.7% ($n = 5$) and between day precision in the range ±3.3 to ±9.7%. The method was linear over the range of 10–400 ng/ml of indapamide in blood ($R = 0.999$). The LOQ (s/n = 10) of the method was 10 ng/ml. The method was applied in a study of the pharmacokinetics and bioequivalence of generic indapamide capsules (2.5 mg) in comparison with reference indapamide tablets (2.5 mg), in 20 healthy male Chinese volunteers. The mean values of major pharmacokinetic parameters of C_{max}, $AUC_{0–48}$, $AUC_{0–\infty}$, T_{max}, and $t_{1/2}$ of indapamide in healthy male Chinese volunteers after *po* a single 5 mg dose for the test product were 331.0 ± 39.2 ng/ml, 6193.7 ± 873.5 ng h/ml, 7311.8 ± 1232.3 ng h/ml, 3.2 ± 0.9 h, and 17.3 ± 2.8 h, respectively. There was no significant differences between the two formulations.
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1. Introduction

Indapamide (4-chloro-*N*-(2-methyl-1-indolinyl)-3-sulphamoyl benzamide) is a non-thiazide indole derivative of chlorosulphonamide, has an anti-hypertensive action causing a drop in systolic, diastolic and mean blood pressure. This anti-hypertensive action is maximal at a dose of 2.5 mg/day and the diuretic effect is slight, usually without clinical manifestation. At higher doses, the diuretic effect becomes more prominent. The extra-renal antiaction of 2.5 mg/day is demonstrated as a decrease in vascular hyperactivity and a reduction in total peripheral and arteriolar resistance. The extra-renal mechanism of action has also been demonstrated by the maintenance of the anti-hypertensive effect in functionally anephric patients. The extra-renal action is thought to be due to the inhibition of transmembrane ionic influx, essentially that of calcium, and the stimulation of the synthesis of the vasodilatory hypotensive prostaglandin PGE2 [1].

Indapamide is widely distributed throughout the body, with extensive binding to some specific sites. In blood, it is highly bound to red blood cells (80%) and, more specifically, to carbonic acid anhydrase (98%) without having any significant inhibiting activity on this enzyme. In plasma, it is relatively highly bound to plasma proteins (79%). It is also taken up to a significant degree in the vascular compartment, the drug has a relatively low apparent volume of distribution

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(approximately 60 l) and 40% of the dose is located in the blood 1 h after administration [2].

A number of HPLC assay methods for indapamide in biological fluids have been reported. The liquid–liquid extraction method Choi et al. [3] for whole blood samples was tedious with UV detection, and control blank was needed for accurate sample analysis [4]. Miller et al. [5] established a much simplified liquid extraction procedure coupled with a HPLC-UV method using an in-house prepared Nucleosil C18 column. Schiavi et al. [6] performed the determination with amperometric detection following a one step liquid–liquid extraction and investigated the pharmacokinetics of sustained and immediate release formulations. Zendelovska et al. [7] recently reported a HPLC-UV method after SPE of whole blood samples. Although SPE is easily automated, good recovery from SPE cartridges is not always guaranteed and the eluting solvent may have to be evaporated prior to reconstitution in HPLC mobile phase.

Although the pharmacokinetics and clinical pharmacology of indapamide have been extensively studied, there is little data from Chinese subjects. Therefore, a new selective HPLC method with UV detection for the determination of indapamide in whole blood samples after a one step liquid–liquid extraction was developed, in order to carry out a pharmacokinetic and bio-equivalence study of two immediate release indapamide formulations in healthy male Chinese volunteers.

2. Materials and methods

2.1. Reagents

The Indapamide and glipizide reference substances were purchased from the National Institute for the Control of Pharmaceutical and Biological Products, Tiantanxili No. 2, Beijing, China. The solvents for HPLC analysis were of HPLC grade purchased from Tedia Company Inc., Fairfield, OH, USA. The water was prepared with double distillation. All other chemicals and reagents were of analytical grade from the Nanjing Chemical Reagent Company, Yanyao Rd, Nanjing, China.

2.2. Subjects

Twenty healthy male Chinese volunteers were selected as subjects. All subjects gave written consent to their participation after having been informed by the medical supervisor about the aim, course and possible risks of the study. The study protocols were approved by the relevant Ethical Review Committee in accordance with the principles of the Declaration of Helsinki, and the recommendations of the State Food and Drug Administration of China.

2.3. Formulations

The test preparation was indapamide capsules, lot no.: 030707, supplied by Zhuhai Rundu Pharmaceutical Co., Guangdong, China. The reference preparation was indapamide tablets, lot no.: 0302015, manufactured by Tianjin Li-sheng Pharmaceutical Co. Ltd., China. Both preparations had a strength of 2.5 mg of indapamide.

2.4. Study design

The volunteers participated in a single dose fasting crossover bio-equivalence study with a one-week interval between each administration. Subjects were admitted to the study clinical unit the evening before the day of each administration and remained under close medical supervision during the study. Each subject received two indapamide test capsules or reference tablets in a randomized order. Subjects fasted 10 h before until 4 h after drug administration. Venous blood samples about 4 ml were collected into heparinized polypropylene tubes at pre-dose and 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0, 16.0, 24.0, 36.0, and 48 h *po* administration and stored at −20 °C until analysis.

2.5. High-performance liquid chromatography

The Agilent 1100 series HPLC system with binary hi-pressure pump, on-line vacuum de-gasser, auto-sampler and a variable wavelength detector was used (Agilent, CA, USA). An Inertsil ODS-3 analytical column (25 cm × 4.6 mm i.d., 5 μm, GL Sciences Inc., Tokyo, Japan) was employed for the separation. The mobile phase was delivered at 1.0 ml/min and consisted of 55 volumes of phosphate buffer solution (a solution of 2 g of potassium dihydrogen phosphate, 3 ml of phosphoric acid, and 3.5 ml of triethylamine in 1000 ml of water), 40 volumes of acetonitrile and 5 volumes of methanol in isocratic mode for 12.5 min, then a gradient flush in 0.5 min from the isocratic to a mixture of 20% isocratic mobile phase and 80% methanol for a further 3 min before equilibrating for next injection. The UV detection was carried out at 240 nm.

2.6. Sample extraction

Whole blood sample of 1.0 ml was spiked with glipizide as internal standard (50 μl of 12 μg/ml glipizide methanol solution) and mixed briefly with 0.5 ml of 0.1 mol/l potassium dihydrogen phosphate solution, then extracted with 5.0 ml of diethyl ether by vigorous vortex-mixing for 3 min and centrifugation at 3000 × *g* force for 10 min. Four milliliters of the supernatant diethyl ether layer was removed, and then evaporated under a gentle stream of nitrogen gas. The residue was reconstituted in 100 μl of a mixture of acetonitrile–methanol (8:1), and then mixed with 50 μl of the phosphate buffer solution used in the LC mobile phase. An aliquot of 20 μl was injected onto the HPLC column.

2.7. Validation and calibration

The calibration curves were constructed routinely for spiked whole blood containing 10, 20, 40, 100, 140, 200,

300, and 400 ng/ml of indapamide during the process of validation and during the study. Precision was assessed as follows: Bias (%) = ((concentration added − concentration found)/concentration added) × 100.

2.8. Pharmacokinetics and statistical analysis

A non-compartmental pharmacokinetic analysis was performed for each of the treatments and for each formulation. Parameters obtained from direct observation of the data include C_{max} and T_{max}. The area under the whole blood concentration–time curve (AUC) from the time of drug administration to the last blood sampling time ($AUC_{0-\tau}$) was calculated according to the linear trapezoidal rule. The terminal elimination rate, λ_z, was estimated by log-linear regression on the last five points, the terminal half-life was calculated with $t_{1/2} = 0.693/\lambda_z$ accordingly, and the $AUC_{0-\infty}$ was the corresponding area extrapolated to infinity by $AUC_{0-\tau} + C_\tau/\lambda_z$. The practical pharmacokinetic program Version 1.0 (3P97) of the Chinese Society of Mathematical Pharmacology (Beijing, China) was used for ANOVA at 90% significance level with logarithmically transformed C_{max} and AUC, and the 90% confidence intervals were calculated.

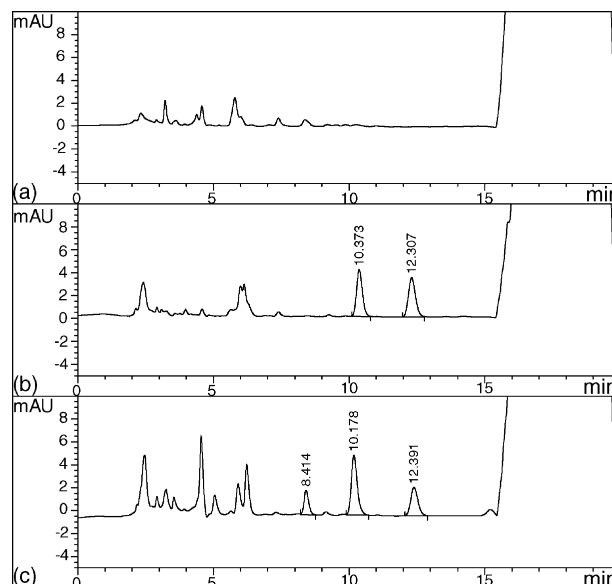

Fig. 1. Representative chromatograms of: (a) blank plasma, (b) plasma spiked with 200 ng/ml indapamide (t_R = 10.4 min) and 600 ng/ml glipizide (t_R = 12.3 min), and (c) plasma sample from a subject 4 h after administration of 5 mg of indapamide in tablet form, the concentration of indapamide was 356.3 ng/ml.

3. Results and discussion

The Inertsil ODS-3 analytical column and the mobile phase used for the assay provided a well defined separation between the drug, internal standard and endogenous components. Typical chromatograms are shown in Fig. 1, the retention times for indapamide and glipizide were about 10.3 and 12.3 min, respectively. There were no interferences from the endogenous components especially the peak at about 8.4 min, which was a serious interfering component on most of the other commercial RP-HPLC columns tested. Quantification was performed using peak area ratios of the drug to internal standard.

The mean absolute recovery of the extraction was determined by comparing the peak area obtained from the blood sample with peak area obtained by the direct injection of pure drug standard solution at three different concentration levels. The mean recoveries of indapamide and glipizide (internal standard) were over 90 and 70%, respectively (Table 1).

The method had within day precision values in the range ±1.2 to ±9.7% (n = 5) and between day precision in the range ±3.3 to ±9.7%. Accuracy expressed in terms of bias was in the range −3.9 to +3.3% (Table 2). The method was linear over the range of 10–400 ng/ml of indapamide in blood (R = 0.999). The LOQ for indapamide was about 10 ng/ml.

The whole blood concentration–time curves of two indapamide products in 20 healthy male Chinese volunteers who received a single 5 mg oral dose in crossover design were compared and the mean curves are shown in Fig. 2. The mean values of the major pharmacokinetic parameters of C_{max}, AUC_{0-48}, $AUC_{0-\infty}$, T_{max}, and $t_{1/2}(\lambda_z)$ of indapamide in healthy male Chinese volunteers after *po* a single 5 mg dose for the test product are shown in Table 3. There were no significant differences between the two formulations on

Table 1
Recovery of indapamide and glipizide from whole blood

Blood concentration (ng/ml)	Recovery (%)					Mean	R.S.D. (%)
Indapamide							
40.0	87.6	85.4	100.2	84.4	106.5	92.8	10.7
140.0	118.2	109.3	100.4	102.4	118.2	109.7	7.7
400.0	111.9	103.5	104.5	111.6	108.4	108.0	3.6
Glipizide							
600.0	70.4	74.2	75.0	74.3	75.8	73.9	2.8

Table 2
Inter- and intra-day precision and bias for the determination of indapamide in whole blood samples($n = 5$)

	Concentration added (ng/ml)		
	40.0	140.0	400.0
Within day			
Concentration found	40.1	134.1	413.2
	35.8	135.5	412.3
	42.7	133.0	416.9
	33.7	133.0	418.4
	40.7	136.9	405.7
Average	38.6	134.5	413.3
R.S.D. (%)	9.7	1.3	1.2
Bias (%)	−3.5	−3.9	3.3
Between days			
Concentration found	32.7	125.5	411.3
	42.7	124.1	379.7
	34.1	134.9	412.3
	39.6	146.1	413.2
	46.4	140.7	414.6
	46.4	138.2	409.2
Average	40.3	134.9	406.7
R.S.D. (%)	14.8	6.4	3.3
Bias (%)	0.8	−3.6	1.7

Bias (%) = ((concentration added − concentration found)/concentration added) × 100.

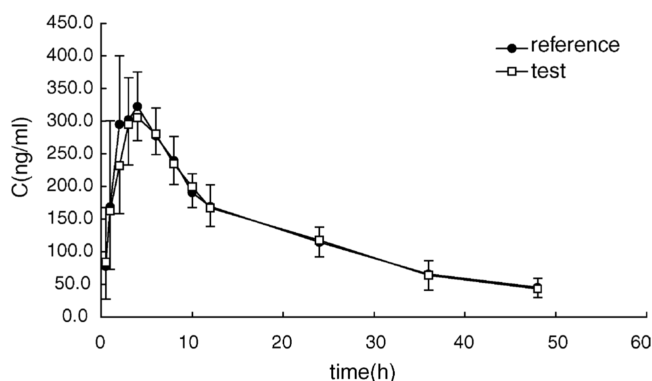

Fig. 2. Mean whole blood indapamide concentration–time profiles following administration of capsules or tablets containing 5 mg of indapamide to 20 healthy subjects, error bars represent S.D.

Table 3
Pharmacokinetic parameters (mean ± S.D.) of indapamide in healthy male Chinese volunteers after *po* a single 5 mg dose of reference tablets and test capsules

Parameters	Reference tablets	Test capsules
C_{max} (ng/ml)	358 ± 43	331 ± 39
T_{max} (h)	2.7 ± 1.0	3.2 ± 0.9
$t_{1/2}(\lambda_z)$ (h)	18.3 ± 3.1	17.3 ± 2.8
$AUC_{0-\tau}$ (ng h/ml)	6290 ± 899	6194 ± 873
$AUC_{0-\infty}$ (ng h/ml)	7529 ± 1323	7312 ± 1232
Relatative bioavailability (%)	–	99.1 ± 11.6

Table 4
Bioequivalence statistics (two one-sided *t*-tests)

Dependent variable	SS	T1	T2	90% Confidence interval (%)
Ln (C_{max})	0.114	14.25	17.32	89.4–96.5
Ln ($AUC_{0-\tau}$)	0.054	12.03	13.83	95.6–101.4

the basis of assessment by a two one-sided *t*-test of the data obtained with logarithmically transformed C_{max} and AUC. The 90% confidence intervals of test to reference ratio of the $AUC_{0–48}$, were within the bio-equivalence criteria range of 80–125%, and that of C_{max} was within 70–143%. Therefore, two products were concluded to be bio-equivalent (Table 4).

4. Conclusions

The selective separation of indapamide from the endogenous components was achieved on an Inertsil ODS-3 analytical column, which was essential for indapamide determination free from interference in human whole blood samples after a single step liquid–liquid extraction sample preparation. The method was successfully applied in a bio-equivalence study. The major pharmacokinetic parameters of indapamide obtained in Chinese volunteers are very important for its optimal clinical usage.

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