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High-performance liquid chromatographic method for determination of clinofibrate and its application to a pharmacokinetic study in healthy volunteers

Li Jian-kang[a,1], Song Ying[a,1], Wang Lei[a,1], Chen Minchun[a], Song Wei[a], Li Xueqing[a], Sun Xiao-li[a], Li Guangqing[b], Jia Yan-yan[a,*], Wen Ai-dong[a,*]

[a] Department of Pharmacy, First Affiliated Hospital of the Fourth Military Medical University, Changle West Street 15, Xi'an, Shaanxi 710032, China
[b] Dima Outai Technology Development Center, Beijing 100029, China

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ABSTRACT

A convenient and rapid HPLC method was developed for the determination of clinofibrate in human plasma using simple protein precipitation with the mixture of acetonitrile and 1 M hydrochloric acid (95:5, v/v) followed by separation using an Inspire C_{18} column with isocratic elution. The detection wavelength was 232 nm and the flow rate was 1.0 ml/min. The mobile phase consisted of acetonitrile and water containing 0.4% ortho-phosphoric acid (73:27, v/v). Linear calibration curve was obtained over the concentrations ranging from 0.5 μg/ml to 32 μg/ml (r^2 = 0.999) with LLOQ of 0.5 μg/ml. The RSD in both the intra-run and inter-run precision study was less than 5.4% and the extraction recoveries were above 90.7%. The HPLC method is reproducible and suitable for the quantification of clinofibrate in plasma. This method was successfully applied to the pharmacokinetic studies of clinofibrate in healthy volunteers. The elimination half-lives ($t_{1/2}$) were (20.47 ± 3.44), (18.19 ± 2.62) and (21.51 ± 4.78) h after single oral administration of 200, 400 and 600 mg clinofibrate, respectively. The results of WinNonlin software showed that the area under the plasma concentration versus time curve from time 0 to 72 h ($AUC_{0–72}$) and peak plasma concentration (C_{max}) were linearly related to dose ($P > 0.05$).

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1. Introduction

With the development of society, dyslipidemia is becoming more common in our life. Approximately 160 million people in China (10% of the population) suffer from hyperlipidaemia (HLP), so hyperlipidaemia is one of the most common chronic diseases there. Hyperlipidaemia represents a determinant for the development of atherosclerosis and as an important risk factor for morbidity and mortality due to cardiovascular disease and stroke, particularly in the context of the insulin resistance syndrome [1].

Clinofibrate, 2,2′-(4,4′-cyclohexylidene diphenoxy)-2,2′-dimethyldibutilic acid (Fig. 1A), is a fibric acid derivative, safe and well tolerated and effective for all types of HLP. The lipid-lowering effect of clinofibrate is ten times higher than that of clofibrate. Clinofibrate can reduce the very low density lipoprotein (VLDL), triglyceride and cholesterol of low density lipoprotein (LDL) and also increase the high density lipoprotein (HDL) through inhibition of the intrahepatic synthesis of triglyceride cholesterol, the biological transformation process of fatty acids, mevalonate, and activation of lipoprotein enzyme, lecithin-cholesterol acyltransferase [2,3]. Clinofibrate acts as the peroxisome proliferator-activated receptor alpha ligand to regulate the transcription of large number of genes that affect lipoprotein and fatty acid metabolism. It is suggested that clinofibrate may induce beta-oxidation by peroxisome and increased H_2O_2 production, which led to augmented ethanol metabolism by catalase [4]. In addition to its beneficial effect on the serum lipid profile, clinofibrate can effectively reduce the plasma fibrinogen level [5,6], but it did not show strong inhibition of human carboxylesterase [7] and any synergistic pro-apoptotic effect of stains in IM-9 human lymphoblasts [8].

The clinical benefit of fibrates, such as clofibrate, gemfibrozil, bezafibrate and fenofibrate favoured in the treatment of hyperlipidaemia is limited to patients with renal failure, because these fibrates are excreted from the kidney. Studies reported occurrence of adverse effects including rhabdomyolysis and elevated serum creatine phosphokinase (CPK) due to accumulation of the drugs [9–11]. Clinofibrate is excreted mostly from the bile [12]. So clinofibrate will be more effective in patients with continuous ambulatory peritoneal dialysis (CAPD) [13] or renal failure.

Clinofibrate first came into the market in 1981 in Japan (Sumitomo Pharmaceutical Co., Ltd.). It is official in the Japanese

* Corresponding authors. Tel.: +86 29 84775475; fax: +86 29 84773636.
E-mail addresses: jiayanyan-2004@hotmail.com (J. Yan-yan), adwen-2004@hotmail.com (W. Ai-dong).

[1] These authors contributed equally to the completion of this study and the writing of this paper.

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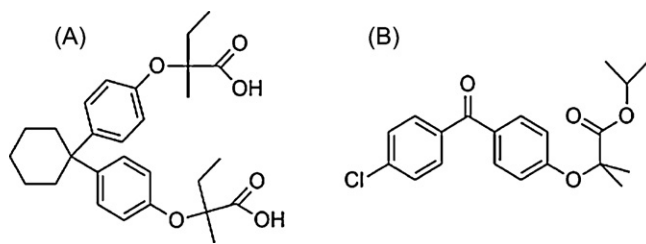

Fig. 1. The chemical structure of clinofibrate (A) and fenofibrate (B).

Pharmacopoeia 16 [14]. Clinofibrate which was produced later also by other pharmaceutical companies of Japan has been a kind of prescription drugs in hospitals in Japan. The technology of the production of clinofibrate tablets was patented in 2010 by Tianjin Han Kang Pharmaceutical Biotechnology Co., Ltd. (China) and was transferred later to XinTong pharmaceutical Co., Ltd. (China). Recently, clinofibrate tablets have been approved to conduct clinical trial in China. As little information is available of the pharmacokinetic characteristics of clinofibrate in human, the aim of our study was to assess the pharmacokinetic properties of clinofibrate in healthy volunteers.

No method has been reported for the determination of clinofibrate in plasma. Under similar analytical conditions to those used for fenofibrate [15] and bezafibrate [16], a HPLC method was developed, which was efficient in analyzing large numbers of plasma samples obtained from pharmacokinetic studies after oral administration of clinofibrate.

2. Materials and methods

2.1. Chemicals and reagents

Clinofibrate (99.5% purity) was supplied from XinTong pharmaceutical Co., Ltd. and the internal standard (I.S.), fenofibrate (100% purity) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Acetonitrile of HPLC grade was purchased from Fisher Scientific (Pittsburgh, PA, USA). Ortho-phosphoric acid and hydrochloric acid were of analytical grade from Tianjin Tianli Chemical Reagent Co., Ltd. (Tianjin, China). Purified water from a Milli-Q system (Millipore, Bedford, MA, USA) was used throughout the experiment. Drug-free human plasma from healthy donors was kindly provided by the Blood Center of Xijing Hospital (Shaanxi, China) and stored at −20 °C.

2.2. Instrumentation and chromatographic conditions

Analyses were performed on an Agilent 1200 series (Agilent Technologies, USA) liquid chromatographic system which was composed of a binary pump, a variable wavelength detector, an autosampler and a computer system for data acquisition (Agilent Chemstation). The analytical column employed was a DIKMA Inspire C_{18} column (150 mm × 4.6 mm I.D., 5 μm particle size) protected with a ODS guard column (10 mm × 4.6 mm I.D., 5 μm particle size). The detection wavelength was set at 232 nm and the flow rate was 1.0 ml/min. The column oven temperature was set at 35 °C. The mobile phase consisted of acetonitrile and water containing 0.4% ortho-phosphoric acid (73:27, v/v).

2.3. Preparation of the stock and standard solutions

Stock solution of clinofibrate was prepared in acetonitrile at a concentration of 1 mg/ml. The internal standard stock solution was prepared by dissolving 10 mg fenofibrate in 100 ml acetonitrile. Clinofibrate stock solution was then diluted with acetonitrile to obtain clinofibrate concentrations of 400, 200, 80 and 10 μg/ml. Standard solutions of clinofibrate at concentrations of 160, 80, 40, 20, 10, 5 and 2.5 μg/ml were prepared by serial dilution of clinofibrate stock solution with acetonitrile. All stock solutions were kept at −20 °C and brought to room temperature before use.

2.4. Sample preparation

The 200 μl aliquot of plasma sample was mixed with 20 μl I.S. (100 μg/ml) in a 1.5 ml tube. The mixture was vortexed for 30 s, proteins precipitated with 600 μl acetonitrile and 1 M hydrochloric acid (95:5, v/v), vortexed for 3 min, and then centrifuged at 16,000 × *g* for 10 min at room temperature. 20 μl aliquot of the supernatant fraction was injected into the HPLC system for analysis.

2.5. Preparation of calibration curves and quality control plasma samples

Calibration curves of clinofibrate were prepared at concentration levels of 0.5, 1.0, 2.0, 4.0, 8.0, 16 and 32 μg/ml by spiking appropriate amounts of the standard solution in 200 μl blank human plasma. Three levels of clinofibrate quality control (QC) samples were prepared in blank human plasma at the nominal concentrations of 1.25, 6.0 and 20 μg/ml. All samples were stored at −20 °C until analysis. One set of standards and quality controls were analyzed on each analysis day with the same procedure for plasma samples as described above.

2.6. Method validation

Complete method validation of clinofibrate determination in human plasma was done following the FDA guideline for validation of bioanalytical methods (US Food and Drug Administration, Center for Drug Evaluation and Research, 2001) [17]. The method was validated for selectivity, linearity and lower limit of quantification, accuracy and precision, recovery and stability.

2.6.1. Selectivity

The chromatograms of drug-free human plasma from six healthy donors were compared. Each blank plasma sample was tested using the protein precipitation procedure to ensure that no endogenous peaks co-eluted with clinofibrate or I.S.

2.6.2. Accuracy and precision

In order to assess the accuracy, intra-run and inter-run precisions, replicate measurements of the QC samples were evaluated. The intra-day precision was evaluated through the QC samples analyzed five times a day. The same procedure was performed once a day for three consecutive days to determine inter-day precision. The precision was calculated by using the relative standard deviation (RSD) and the accuracy was expressed as relative error (RE). The precision and accuracy were considered acceptable within ±15%.

2.6.3. Linearity and lower limit of quantification

Duplicate seven-point standard curves were run on three separate days to assess the linearity. The curves were fitted by a weighted ($1/x$) least-squares linear regression method through the measurement of the peak area ratio of the analyte to I.S. The acceptance criterion of correlation coefficient for a calibration curve was 0.999 or better and each back-calculated standard concentration must be within 15% deviation except at the lower limit of quantitation (LLOQ) which was within 20%. LLOQ was the lowest concentration of the standard curve that could be measured with acceptable accuracy and precision. Both of the RSD ($n = 6$) and RE were within ±20%.

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