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Short Communication

Determination of 2,2-dimethyl-N-(2,4,6-trimethoxyphenyl)-dodecanamide, a lipid regulator, in rat plasma and mesenteric lymph by reversed-phase high-performance liquid chromatography

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ABSTRACT

2,2-Dimethyl-N-(2,4,6-trimethoxyphenyl)dodecanamide (I, CI-976) has been determined in rat mesenteric lymph and plasma using a rapid and sensitive high-performance liquid chromatographic method. The samples prepared from plasma and lymph by liquid–liquid extraction were analysed on a reversed-phase C_{18} column using isocratic conditions and ultraviolet detection. The method was applied to the determination of levels of I in Wistar rats after intraduodenal administration of 110 mg/kg of I as a lipid emulsion.

INTRODUCTION

2,2-Dimethyl-N-(2,4,6-trimethoxyphenyl)dodecanamide (I, CI-976) (Fig. 1) is a lipid regulator which competitively inhibits the enzyme acyl-CoA:cholesterol acyltransferase (ACAT). ACAT catalyzes esterification of cholesterol within intestinal mucosal cells and arterial intima and consequently is important in cholesterol absorption as well as accumulation in arteries [1]. This work describes reversed-phase high-performance liquid chromatographic (HPLC) methods for the determination of I in Wistar rat plasma and mesenteric lymph. A simple extraction technique for I was developed for plasma and lymph and chromatographic conditions were selected to provide adequate sensitivity. The methods have been successfully applied to pharmacokinetic studies in Wistar rats.

EXPERIMENTAL

Chemicals

Compound I was synthesized by the Parke-Da-

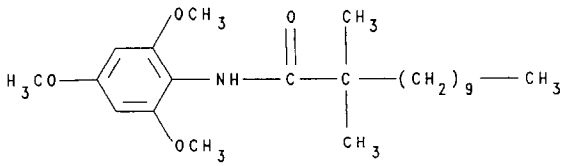

Fig. 1. Structure of CI-976 (I).

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vis Pharmaceutical Research Division, Warner Lambert Company (Ann Arbor, MI, USA). Isopropanol, methanol, acetonitrile and water were all obtained as HPLC grade from Fisher Scientific (Fairlawn, NJ, USA). Blank plasma and mesenteric lymph were collected from fasted male Wistar rats.

Standard preparations

A stock solution of I for lymph standards (1 mg/ml) was prepared by weighing 100 mg of the powder in a 100-ml volumetric flask and diluting the compound to volume with methanol. For working standards, a 100 μg/ml stock solution was prepared by diluting 1 ml of this solution to 10 ml with methanol. Appropriate dilutions of the stock solution were made in methanol for spiking blank rat lymph or plasma. Aliquots (100 μl) of the appropriately diluted stock solution of I were added to 900-μl amounts of blank rat plasma or mesenteric lymph to yield working standards of the desired concentrations.

Sample preparation

Plasma and lymph samples (100 μl) were placed in 1.5-ml polypropylene microcentrifuge tubes and 300 μl of methanol were added. The tubes were vigorously vortex-mixed for 1 min, allowed to stand for 10 min and vortex-mixed again for 30 s. The tubes were centrifuged at 16 000 *g* for 10 min and the supernatants carefully transferred to glass HPLC vials for assay.

Instrumentation

An automated HPLC system was used, consisting of a HP 1090M Chemstation equipped with a Model 35900 A/D interface (both from Hewlett-Packard, Avondale, PA, USA) and a Kratos Spectroflow 757 ultraviolet (UV) detector (ABI, Ramsey, NJ, USA) operated at 230 nm for plasma. A detection wavelength of 250 nm was used for lymph in order to eliminate interference seen in this biological fluid. A Beckman Ultrasphere ODS 5-μm column (stainless steel, 250 mm × 2 mm I.D.) equipped with a Brownlee ODS 5-μm guard cartridge (30 mm × 2.1 mm I.D., Rainin Instruments, Woburn, MA, USA) and maintained at 23 ± 3°C was employed for all analyses. Samples were chromatographed isocratically at a flow-rate of 0.9 ml/min for plasma (pressure, 27.5 MPa) and 0.5 ml/min for lymph (pressure, 28.8 MPa). The mobile phase used for plasma consisted of a mixture of acetonitrile, methanol and water (6:3:2, v/v). For lymph, a mixture of isopropanol, acetonitrile and water (153:153:94, v/v) was used in order to achieve adequate separation of I from the different endogenous components found in lymph. Degassing of mobile phase was achieved by helium sparging.

Calibration and precision

Five calibration standards for plasma containing 0.25, 0.50, 1, 2 and 5 μg/ml and eight standards for lymph containing 0.30, 0.50, 1, 5, 10, 20, 40 and 50 μg/ml were processed daily with each set of unknowns. Calibration curves were constructed by plotting peak areas of I as a function of the concentration of I. The best-fit straight line was determined using least-squares linear regression. To determine the precision of the assay procedures, triplicate plasma and lymph standards were analyzed in triplicate on three separate days to yield nine replicate values for each of the five plasma and eight lymph concentrations.

Assay recovery

The percentage recovery of I from plasma and lymph was assessed for three concentrations of the respective calibration standards. For plasma, 0.25, 1 and 5 μg/ml concentrations were used; for lymph, 0.30, 5 and 50 μg/ml concentrations were used. The procedure consisted of extracting three samples (100 μl) containing I and performing triplicate injections of each sample. Three injections of the same amounts of I in the appropriate mobile phase were injected directly. The peak areas of I at each concentration were measured. The recovery of I was computed using the following equation:

$$\text{recovery}\,(\%) = \frac{\text{mean peak area of extract}}{\text{mean peak area of direct injection}}$$

RESULTS AND DISCUSSION

Assay linearity, precision and accuracy

The relationship between peak area and concentration of I was linear over the concentration ranges studied (0.25–5 μg/ml for plasma and 0.30–50 μg/ml for lymph) and yielded a correlation coefficient of 0.999 or greater for both assays. The precision of the plasma assay ranged from 2.83 to 7.30%, and accuracy over the concentration range studied ranged from −0.8 + 7.6%. The presence of I as a rider peak on the plasma impurity front resulted in a slight decrease in the accuracy of quantitation of I in plasma as compared to lymph. The precision of the lymph assay over the concentration range studied ranged from 0.95 to 11.8%, with accuracy ranging from −0.01 to +2.95%. The limit of quantitation for the assay was defined as the concentration of I per millilitre of plasma or lymph at which the signal-to-noise ratio was 3. For plasma, the limit of quantitation was 0.20 μg/ml and for lymph, 0.30 μg/ml.

Selectivity

Representative chromatograms obtained from plasma and lymph analyses are depicted in Figs. 2 and 3, respectively. Retention times for CI-976 in plasma and lymph are 5.3 and 4.2 min, respectively. Absence of interfering peaks eluting at times similar to I gave assurance that I peaks were adequately resolved from other plasma or lymph components. Over the concentration ranges studied, plasma recovery varied from 103.56 to 107.60% and lymph recovery varied from 98.81 to 102.08%.

The described methods have been successfully applied to pharmacokinetic and gastrointestinal absorption studies in Wistar rats following intraduodenal administration of single doses of I as a 20% oil-in-water emulsion (Figs. 4 and 5). Blood was collected into heparinized tubes from an indwelling jugular venous cannula and the plasma harvested by centrifugation. Lymph was collected into heparinized tubes from an indwelling cannula located in the superior mesenteric lymph duct. The concentrations of I ranged from 0.64 to 2.68

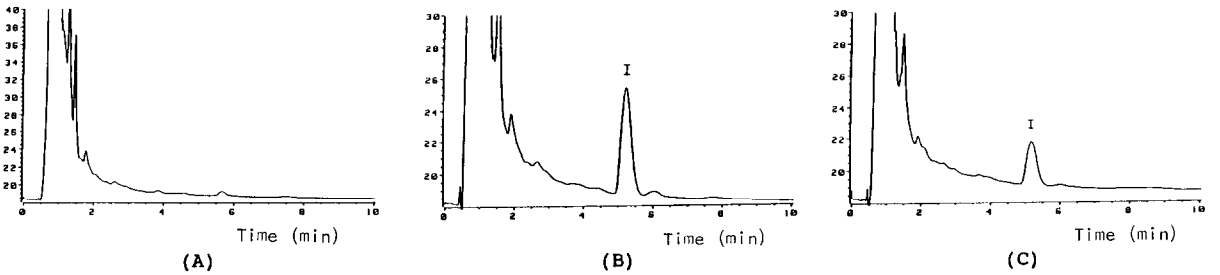

Fig. 2. Chromatograms of (A) blank rat plasma, (B) rat plasma spiked with 5 μg/ml of I, and (C) rat plasma 45 min following intraduodenal administration of 25 mg of I as a lipid emulsion.

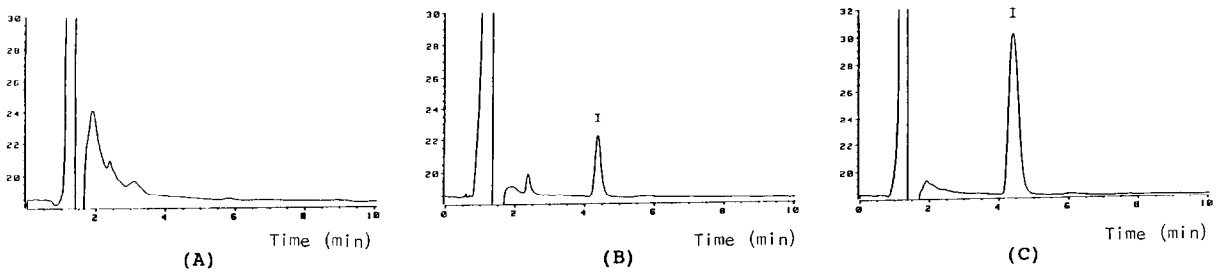

Fig. 3. Chromatograms of (A) blank rat mesenteric lymph, (B) rat mesenteric lymph spiked with 5 μg/ml of I, and (C) rat mesenteric lymph 60 min following intraduodenal administration of 25 mg of I as a lipid emulsion.

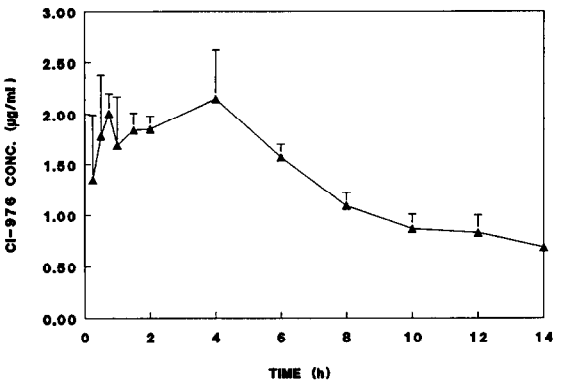

Fig. 4. Concentration of I (CI-976) in rat plasma *versus* time following intraduodenal administration of 110 mg/kg of I as a 20% lipid emulsion (error bars represent standard deviations, $n = 3$).

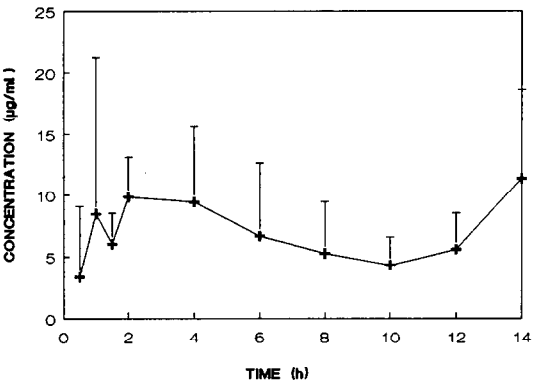

Fig. 5. Concentration of I in rat mesenteric lymph *versus* time following intraduodenal administration of 110 mg/kg of I as a 20% lipid emulsion (error bars represent standard deviations, $n = 3$).

µg/ml in plasma and from 0.47 to 23.17 µg/ml in lymph.

In conclusion, a selective and simple method has been developed for I in plasma and lymph for evaluating the gastrointestinal absorption of I in rats.

REFERENCE

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