Determination of Gallopamil in Human Plasma by Selected Ion Monitoring

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A gas chromatographic mass spectrometric procedure using selected ion monitoring is described for the quantification of gallopamil in human plasma. Gas chromatographic separation of gallopamil from phenolic metabolite isomers was made possible by treatment with ethyl chloroformate. The detection limit for the quantitation by the present method is 0.09 ng/ml of plasma. The method has sufficient sensitivity to permit pharmacokinetic studies with human subjects following the oral administration of gallopamil hydrochloride.

Keywords gallopamil; gas chromatography-selected ion monitoring; extraction—derivationization; oral administration; human plasma

Gallopamil, DL-5-[3,4-dimethoxyphenylethyl]methylamino]-2-isopropyl-2-(3,4,5-trimethoxyphenyl)valeronitrile (a) (Chart 1), is a calcium channel antagonist, about three to five times more potent than verapamil in its cardiovascular parameters.1

High-performance liquid chromatographic methods were reported for the determination of gallopamil in biological fluids with detection limits of 0.22) and 0.9 ng/ml.3) With these methods, it should thus be possible to clarify the pharmacokinetics at a dose of 50 mg in humans. However, a more sensitive means is necessary to determine the pharmacokinetics of gallopamil even at the low dosage of 12.5 mg which might be the lowest clinical dosage for Japanese. This paper describes a selective and sensitive gas chromatographic mass spectrometry selected ion monitoring (GC/MS-SIM) method for the analysis of gallopamil in human plasma. The sample preparation involves extraction—derivatization with ethyl chloroformate and purification by a Sep-pak silica cartridge.

Experimental

Materials and Reagents Gallopamil (a), DL-5-[3,4-isopropyl-2-(3,4,5-trimethoxyphenylethyl)methylamino]-2-(3,4,5-trimethoxyphenyl)valeronitrile (b), used as an internal standard (I.S.), DL-5-[4-hydroxy-3-methoxyphenylethyl)methylamino]-2-isopropyl-2-(3,4,5-trimethoxyphenyl)valeronitrile (c), and DL-5-[4-hydroxy-4-methoxyphenylethyl)methylamino]-2-isopropyl-2-(3,4,5-trimethoxyphenyl)valeronitrile (d) were kindly supplied by Knoll AG (Ludwigs-hafen, G.F.R.). All these compounds were characterized by GC/MS. Ethyl chloroformate was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). The Sep-pak silica cartridge was obtained from Waters Assoc., (Milford, MA, U.S.A.). All other reagents and solvents were of the highest quality available.

Instrumentation A JEOL JMS-DX303 GC/MS instrument equipped with a JMA-DA5000 data processing system was used to determine gallopamil in human plasma. The GC column was a cross-linked methylsilicone fused silica DB-1 megabore column (15 m × 0.53 mm i.d.; coating thickness 1.5 μm, J&W Scientific Inc.). The GC oven temperature was 270°C. The mass spectrometer was operated at an ionization energy of 70 eV with an emission current of 300 μA in the electron impact (EI) mode. The interfacial region between the chromatograph and mass spectrometer was maintained at 270°C. Quantitation was performed by selected ion monitoring, focusing on the common fragment ion at m/z 335 for gallopamil and I.S. Mass spectra were measured with a Shimadzu QP-1000 mass spectrometer by EI and chemical ionization (CI) with isobutane as the reagent gas at a pressure of 4 × 10⁻³ Torr. The ionization energy and trap current were 200 eV and 150 μA, respectively.

Extraction and Derivatization To 1.0 ml of plasma in a round-bottom test tube were added 2.5 ng of a methanolic solution of I.S. and 150 μl of a hydrochloric solution (4N) along with 5 ml of chloroform, and the mixture was then shaken for 15 min at room temperature. After centrifugation at 4000 rpm for 10 min at 18°C, the lower layer was transferred to another identical tube. The extract was vigorously shaken with 450 μl of ethyl chloroformate and 1 ml of sodium hydroxide (1N) for 15 min at room temperature. After centrifugation at 4000 rpm for 10 min at 18°C, the whole of the chloroform extracts was applied onto the Sep-pak silica cartridge column. After being rinsed with 4 ml of chloroform followed by 4 ml of chloroform-methanol (99:1), the cartridge was eluted with 4 ml of chloroform-methanol (95:5). The solvent was evaporated at 40°C using a centrifugal evaporator (Yamato Kagaku Co., Tokyo). To completely remove trace amounts of ethyl chloroformate, the tube was evaporated to dryness under a stream of nitrogen. The dried residue was dissolved in 15 μl of methanol and 1 μl of this solution was subjected to GC/MS.

Calibration Curve The calibration curve for the determination of gallopamil by GC/MS-SIM was obtained by plotting the ratio of the peak area of gallopamil to that of I.S. (2.5 ng/ml of plasma) against concentration. Standard solutions containing 0.09, 0.19, 0.47, 2.35, 7.04 and 14.08 ng/ml of plasma were prepared.

Results and Discussion

GC/MS Conditions Since gallopamil has the same mass spectrometric properties as verapamil41 due to its methoxy analogue structure, the EI mass spectrum of gallopamil showed only one major fragment at m/z 333 (Fig. 1), formed by loss of the dimethoxybenzyl moiety. The situation was the same with the similarly structured I.S., having the same extraction and chromatographic characteristics as gallopamil: it showed a very dominant fragment at m/z 333 due to the loss of the trimethoxybenzyl moiety. The common fragment ion at m/z 333 for gallopamil and I.S. was used for mass chromatography and SIM analysis.

Gallopamil is extensively metabolized in the liver to produce a secondary amine as the main metabolite by N-dealkylation. However, the drug is also susceptible to O-demethylation, yielding the corresponding phenolic metabolite isomers.31 Figure 2A shows a typical mass chromatographic separation of gallopamil and two kinds of O-demethylated metabolites without derivatization reaction. Monitoring the characteristic ion of m/z 333, it is evident that these phenolic metabolites interfered with the measurement of the unchanged drug. Since the metabolites...
have been reported present as minor metabolites in subjects dosed with gallopamil,\textsuperscript{5}) we separated gallopamil from them by selective derivatization of the phenolic hydroxy function. However, ordinary derivatization methods such as acetylation, trifluoroacetylation and silylation were found unsuitable for the determination of gallopamil, due either to inadequate reactivity or instability of the products. The selective conversion of phenolic metabolites to O-carbamates was carried out according to the method of Makita \textit{et al.}\textsuperscript{6,7}) with a minor modification; this was reported as an alternative method for analyzing simple phenols and phenolic acids as their O-isobutylcarbonyl derivatives. As shown in Fig. 2B, use of this O-ethoxy-
standard. Although the baseline in the SIR is relatively high, the peak intensities were sufficient to determine gallopamil, since the drug-free control plasma gave no interfering peaks. The retention times for gallopamil and I.S. were approximately 4.0 and 5.2 min, respectively.

The detection limit was approximately 0.05 ng (as free base)/ml of plasma and the calibration curve was obtained by spiking a sample of plasma with a gallopamil range of over 0.09 to 14.08 ng per ml. As shown in Fig. 4 and Table I, the calibration curve was linear with correlation coefficients of 0.9998 (n = 6), and coefficients of variation (n = 5) of 2.2% on average.

Monitoring of Plasma Concentration It was possible to determine gallopamil at a level as low as 0.09 ng/ml of plasma by the above method without interference from the metabolites. The applicability of this method was confirmed by determination of plasma concentration following the oral administration of 12.5 mg of gallopamil hydrochloride to healthy subjects. A representative plasma concentration level is shown in Fig. 5. At 12 h, the level of gallopamil was 0.1 ng/ml. The sensitivity of this technique is sufficiently high to permit pharmacokinetic studies on man.

Conclusions The chromatographic separation of gallopamil from phenolic metabolites was effected through the formation of O-ethoxyxycarbamate using GC/MS-SIM, without any interference. The results of determinations of plasma concentration following the oral administration of the drug at a dose as low as 12.5 mg confirms the usefulness of this method for human subjects.

References