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Note

Simple method for determination of flunarizine in serum by gas chromatography

AKIRA YAMAJI*, KAZUSABURO KATAOKA, MASAKO OISHI and NOBUHIRO KANAMORI

Department of Pharmacy, Osaka University Hospital, 1-50 Fukushima 1-chome, Fukushima-ku, Osaka 553 (Japan)

and

TETSUZO TAGAWA and TAKASHI MIMAKI

Department of Pediatrics, Osaka University Hospital, 1-50 Fukushima 1-chome, Fukushima-ku, Osaka 553 (Japan)

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Flunarizine (FL) is a calcium entry blocker and is widely used in the treatment of cerebral and peripheral vascular diseases [1]. It has also been reported to have an anticonvulsant effect [1–3]. FL has a long half-life in plasma and an extended effect, and side-effects such as drowsiness, depression and the extrapyramidal signs have been reported [4]. Since FL has long-term use, pharmacokinetic studies and therapeutic monitoring of this drug are necessary for effective therapeutic trials without untoward effects. Therefore, methods for the determination of serum concentrations of FL are required.

The determination of FL in serum and/or plasma has been reported by gas chromatography (GC) with nitrogen-selective detection [5–7] and high-performance liquid chromatography (HPLC) [8,9]. The GC methods were more selective and sensitive than the HPLC methods. However, the previously reported GC methods, although selective, required complicated extraction procedures and a large sample size (1–2 ml of serum) for routine work. We have already reported a GC method for the simultaneous determination of procainamide and N-acetylprocainamide by the combination of a solid-phase extraction column and a nitrogen-selective detector [10].

This paper describes a new method for determination of FL in serum by GC with nitrogen-selective detection. The extraction procedure is simplified by using

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a solid-phase extraction column, and the serum sample required for assay is 250 μl. Using this improved method, the serum concentrations of FL were determined in patients with epilepsy or cerebrovascular diseases who were administered FL tablets orally.

EXPERIMENTAL

Apparatus and chromatographic conditions

A Hewlett-Packard Model 5710A gas chromatograph equipped with a nitrogen–phosphorus detector and a Model 3390A reporting integrator were used. The glass column (2.4 m × 2 mm I.D.) was packed with 3% silicone OV-17 on 80–100 mesh Chromosorb W. The carrier gas was high-purity nitrogen at a flow-rate of 30 ml/min. The detector purge was hydrogen and air at flow-rates of 3.5 and 70 ml/min, respectively. The column temperature was 260 °C (isothermal), and the injector port and detector temperatures were 300 °C. The detector sensitivity was adjusted to an offset of 30% (attenuation 2^6).

Reagents

Reagent-grade sodium hydroxide and methanol and chromato-grade *n*-hexane and ethyl acetate were obtained from Wako (Osaka, Japan). A pure standard of flunarizine hydrochloride was kindly provided by Kyowa Hakko Kogyo (Tokyo, Japan). Cinnarizine, used as the internal standard, was kindly provided by Eisai (Tokyo, Japan).

Analytical procedure

A 250-μl volume of serum was pipetted into a borosilicate glass culture tube (12 × 75 mm), then 25 μl (500 ng/ml) of a methanolic solution of cinnarizine as the internal standard and 750 μl of 0.5 *M* sodium hydroxide were added and mixed well. All the solution was applied to an Extrelut® column (E. Merck, Darmstadt, F.R.G.). After it had stood for 10 min at room temperature, the sample was eluted with 10 ml of *n*-hexane–ethyl acetate (5:1, v/v). The eluate was collected and evaporated to dryness. The residue was dissolved in 10 μl of methanol, and 1–2 μl were injected into the GC system. The calibration curve was prepared by adding to blank human serum.

RESULTS AND DISCUSSION

Albani et al. [9] reported that FL is easily extracted from aqueous solutions at both high (>10) and low (<2) pH. However, we could not obtain sufficient recoveries from acidic and neutral solutions, so we used 750 μl of 0.5 *M* sodium hydroxide. Solutions of 1 *M* and 2 *M* sodium hydroxide gave similar results. As for an organic solvent for extraction and elution of FL from serum samples, dichloromethane, chloroform and diethyl ether were investigated, but recoveries of FL were not satisfactory. Use of ethyl acetate alone gave interfering material from biological fluid, but *n*-hexane–ethyl acetate (5:1) gave adequate recoveries ($95.7 \pm 2.7\%$, $n = 5$) and did not produce interfering substances.

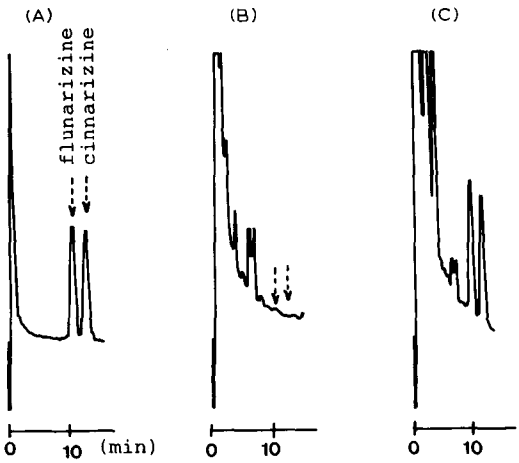

Fig. 1. Gas chromatograms of flunarizine. (A) Standard solution containing flunarizine and cinnarizine (internal standard); (B) extracted from human blank serum; (C) extracted from serum of a patient taking flunarizine, the flunarizine concentration was found to be 53 ng/ml.

Fig. 1 shows chromatograms from a blank serum and serum from a patient taking FL. No interfering peaks were observed in blank serum. Commonly used antiepileptic agents, such as phenobarbital, phenytoin, carbamazepine and valproate, did not interfere in this method. The ratio of the peak height of FL to the peak height of the internal standard was plotted on the y-axis against various concentrations of FL (in ng/ml) on the x-axis: the relation was linear ($r=0.9999$, $y=0.019x-0.03$) over the concentration range 5–200 ng/ml in serum. The limit of quantitation of FL was 1 ng/ml and the detection limit with on-column injection of FL was 50 pg (signal-to-noise ratio = 2). A flame-ionization detector was not useful because the detection limit was 40 ng and the serum background interference was high on the chromatograms.

The accuracy and precision of this method were examined using human serum samples to which were added 5, 10, 50 and 100 ng/ml FL. The overall average accuracy for FL was $98.9 \pm 2.1\%$ ($n=20$). The within-day precision was 1.1% ($n=5$) and the between-day reproducibility was less than 3% over the five days.

Though GC methods [5–7] using nitrogen-selective detection have already been reported, the sample size required was more than 1 ml of serum and the extraction procedures were complicated for routine assay. In the present GC method, the required sample size is only 250 μl of serum and the extraction process is much simpler and more rapid on an Extrelut column. This solid–liquid extraction is more useful than liquid–liquid extraction because FL is obtained quantitatively from serum without gel formation and clean-up is performed at the same time. Additionally, the precision and recovery were improved over the previous GC methods.

This method was applied to a pharmacokinetic study of four patients. They participated after giving written informed consent. The subjects, aged 12 to 17 years, were one epileptic patient (subject B) and three patients with cerebrovascular diseases. Fig. 2 shows the time courses of FL serum concentration after a

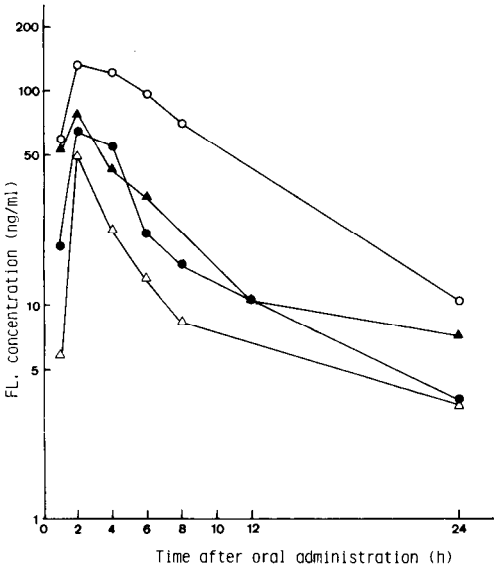

Fig. 2. Serum concentration of flunarizine after a single oral administration to patients. Subject A (▲) 13-year-old female, dose 20 mg per 34 kg body weight. Subject B (△) 17-year-old male, dose 20 mg per 42 kg. Subject C (●) 12-year-old male, dose 20 mg per 42 kg. Subject D (○) 13-year-old female, dose 20 mg per 53 kg.

single oral dose of 20-mg FL tablets. The pharmacokinetic parameters were calculated using the data up to 24 h (Table I). The area under the curve from 0 to 24 h, $AUC_{0-24\,h}$, was calculated by the trapezoidal rule, and the biological half-life ($t_{1/2}$) was calculated by the residual method.

The mean($\pm$S.D.) values of the maximum serum concentration (C_{max}), $AUC_{0-24\,h}$ and $t_{1/2}$ were 81.3 ± 36.0 ng/ml, 709.3 ± 552.7 ng h/ml and 6.2 ± 0.6 h, respectively. We found individual variations in AUC and C_{max} among patients.

The method was also applied to therapeutic monitoring of FL levels in three patients with epilepsy and two patients with cerebrovascular disease who took FL tablets orally. Epileptic patients were coadministered antiepileptic drugs such as phenobarbital, phenytoin, carbamazepine and valproate. Five patients aged from 11 to 20 years were administered FL dosages of 0.18–0.50 mg/kg per day

TABLE I

PHARMACOKINETIC PARAMETERS OF FLUNARIZINE IN PATIENTS

Patient	Dose (mg/kg)	C_{max} (ng/ml)	T_{max} (h)	AUC (ng h/ml)	$t_{1/2}$ (h)
A	0.59	79.1	2	555.0	6.7
B	0.48	49.3	2	283.1	6.6
C	0.48	64.8	2	478.8	5.6
D	0.38	132.1	2	1520.4	5.8

(mean ± S.D. 0.36 ± 0.14 mg/kg per day). The trough concentrations were 6.7–53.8 ng/ml in serum. There was no relation between serum levels and clinical effects in this series. Further studies are necessary to investigate the therapeutic FL levels in epileptic patients.

We conclude that the present GC method is a more selective and sensitive procedure for routine assay of FL in serum. It involves a simpler extraction procedure and a smaller sample size (250 μl), and is considered to be suitable for pharmacokinetic studies and the therapeutic monitoring of FL.

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