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Short communication

Sensitive assay for verapamil in plasma using gas–liquid chromatography with nitrogen–phosphorus detection

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Abstract

A sensitive gas-chromatographic method for quantitative analysis of verapamil in human plasma is described. The method involves a single extraction procedure, followed by separation on a capillary column and detection with a nitrogen–phosphorus detector. The detection limit, based upon an assayed plasma volume of 0.5 ml, is 2 ng/ml. The standard curve is linear in the concentration range of 2 to 1000 ng/ml. The recovery of verapamil by pentane–isopropanol extraction was found to be 95%. Zipeprol is used as the internal standard. No interference from drugs needed for the associated cancer therapy has been found. Serum verapamil concentrations are determined by this method in fourteen cancer patients undergoing treatment with adriamycin.

Keywords: Verapamil

1. Introduction

Verapamil (Fig. 1) is a first-generation calcium-entry antagonist drug that is eliminated by hepatic metabolism [1,2]. The drug is a valuable and widely used agent in the management of essential hypertension and angina [3].

The concurrent administration of verapamil and those drugs needed in therapy is of considerable interest because verapamil can enhance the efficacy of the therapeutic agents. For example, verapamil impairs the metabolism of quinidine to 3-hydroxyquindine and reduces the oral clearance of quinidine to a degree that could be of clinical significance, given the narrow therapeutic index of this drug [3].

Verapamil has been shown to inhibit hepatic monooxygenase activity in rodents [4], and to inhibit the metabolism of antipyrine [3], taxol [5] and theophylline in man [6].

Verapamil has also been known as a multidrug

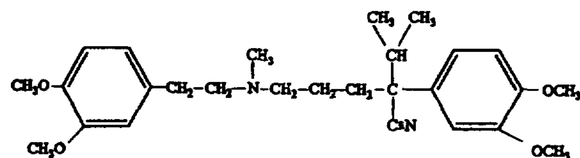

Fig. 1. Structure of verapamil.

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resistance (MDR) modulator. Tsuruo et al. [7] found that verapamil could inhibit *p*-glycoprotein-mediated anticancer drug efflux from cancer cells, resulting in an improved anticancer effect. Since their initial report, the MDR-modulating effect of verapamil has been demonstrated in vitro and in vivo by many researchers. Salmon et al. [8] tested verapamil as a possible drug for the reversal of drug resistance in chemotherapy-refractory multiple myeloma and reported that addition of verapamil could induce 5 partial remissions among 22 patients (23%) refractory to chemotherapy alone [8]. With this encouraging result, we have been testing verapamil as a potential drug capable of reversing drug resistance in chemotherapy-refractory lymphoma patients.

Monitoring of the plasma modulator level is indispensable for the MDR-modulator trial, as the responses and toxicities resulting from administration of modulator should be interpreted in conjunction with the corresponding plasma modulator concentration. The method of measurement should also be sensitive, specific and rapid, so that the rate of drug administration could be adjusted according to the plasma drug level.

Attempts to quantify the concentration of verapamil have predominantly involved high-performance liquid chromatographic separation [5,9–15]. For the specific and highly sensitive determination of verapamil, we suggest a gas chromatographic separation with nitrogen–phosphorus detection (NPD). NPD is an alkali-flame detector. It has good sensitivity to nitrogen (10^{-3} g of N/S), selectivity (10^4 g of N/g of C, 10^5 g of P/g of C), a wide dynamic range (10^5) and it also is quite stable. Because of its simplicity, rapidity and specificity, it may offer general advantages over existing methods [5,9–16].

2. Experimental

2.1. Materials

Verapamil was obtained from the Korea Cancer Center Hospital (Seoul, Korea) and zipeprol was from Dong Shin Phamaceuticals, (Seoul, Korea). The solvents *n*-pentane, methanol and isopropanol (Merck, Darmstadt, Germany) were used.

2.2. Stock solutions

Standard solutions of zipeprol and verapamil (10 μg/ml) were prepared in distilled water and were kept at 4°C. Under these conditions, the solutions were stable for several weeks. Working solutions of 1 and 0.1 μg/ml were prepared by sequential dilution.

2.3. Calibration graph and quantitation

A calibration graph for verapamil was established by adding 1, 5, 25, 50, 100, 250 and 500 ng of verapamil and 200 ng of zipeprol to 0.5 ml of drug-free plasma and processing the mixtures according to the method described below. The ratio of the peak area of verapamil to that of zipeprol was used to calculate the calibration graph for the quantitation of verapamil in plasma samples.

2.4. Extraction procedure

For each analysis, 0.5 ml of plasma was used, to which 20 μl of a 10 μg/ml zipeprol solution were added as an internal standard, in a glass centrifuge tube. Then, 7 ml of pentane–isopropanol (97:3, v/v) and 50 μl of a 5 *M* sodium hydroxide solution were added and the tubes were stoppered and shaken mechanically for 20 min, then centrifuged for 5 min at 800 *g*. The extraction procedure was performed at 25°C. The organic layer was transferred to a 15-ml glass centrifuge tube and evaporated to dryness, at reduced pressure, and then dissolved in 100 μl of methanol, of which 3 μl were injected onto the chromatographic system.

2.5. Gas chromatography

All GC experiments were performed with a Hewlett-Packard (HP) 5890B gas chromatograph with a nitrogen–phosphorus detector, connected to an HP 3392A integrator. All injections were made with an HP 7673A autosampler. GC conditions for the plasma sample analyses are given in Table 1.

2.6. Drug administration and sample collection

Fourteen patients (eight male, six female, median age 42 years) with good cardiovascular function and

Table 1
Gas chromatographic conditions used for the determination of verapamil concentration in plasma

Parameter	Conditions
Column	HP fused-silica capillary, cross-linked 5% phenylmethyl silicone (SE-54); 25 m × 0.2 mm I.D., film thickness 0.33 μm
Injector temperature	310°C
Detector temperature	300°C
Initial temperature	100°C
Programming rate	20°C/min
Final temperature and time	310°C for 10 min
Carrier gas flow-rate	Helium at 0.9 ml/min
Auxiliary gas flow-rate	Helium at 20 ml/min
Hydrogen flow-rate	4.0 ml/min
Air flow-rate	100 ml/min
Splitting ratio	1:10
Septum-purge flow-rate	5.1 ml/min

histologically confirmed Non-Hodgkin's lymphoma refractory to EPOCH chemotherapy were studied. Under the EPOCH regimen, MDR-drugs such as doxorubicin, vincristine and etoposide were administered by a four-day continuous i.v. infusion [17]. Verapamil was administered using an i.v. loading of 0.15 mg/kg body weight, 1 h before MDR-drug infusion, followed by continuous i.v. infusion, at a velocity of 0.2 mg/kg/h. The verapamil dose was escalated every day by 0.05 mg/kg/h up to 0.45 mg/kg/h, unless there was any significant toxicity. The administration of verapamil was stopped 12 h after completion of the MDR-drug infusion. A 5-ml sample of venous blood was collected in a heparinized syringe prior to loading and also daily after infusion of verapamil. The blood samples were centrifuged (1000 *g* for 5 min) and the plasma was separated and stored at −20°C until analysis.

3. Results

The gas chromatogram obtained from a plasma sample to which a known amount of verapamil was added is shown in Fig. 2, together with chromatograms obtained from a plasma blank and from a patient receiving verapamil plus adriamycin treatment.

The peaks of verapamil (17.53 min) and zipeprol (13.50 min) were resolved, and no interfering peak from endogenous substances or from other co-administered drugs such as adriamycin, vincristine and etoposide is present. Because the drugs are non-volatile or non-nitrogen- and non-phosphorus-containing compounds, these cannot be detected with the GC–NPD system.

Examination of typical standard curves, by computing a regression line of the peak-area ratios of verapamil to the internal standard concentrations, using a least-squares fit, demonstrated a linear relationship with correlation coefficients that were consistently greater than 0.999 for verapamil. The line of best fit is $y = 0.428x + 0.008$, where x is the analyte concentration (μg/ml) and y is the peak-area ratio of verapamil to internal standard. The within-run precision and accuracy of the method were determined by assaying 0.5-ml aliquots of plasma (Table 2).

The recovery of verapamil at 200 ng/ml, calculated using zipeprol as the external standard, was found to be 95%.

The absolute sensitivity of the NPD detector was 0.1 ng for verapamil. The practical limit of detection was 2.0 ng/ml for verapamil, based upon an assayed plasma volume of 0.5 ml. These limits were defined by a minimal signal-to-noise ratio of three and a C.V. for replicate determinations of 15% or less.

Preliminary observations, carried out on patients receiving verapamil at continuous dosages, indicate

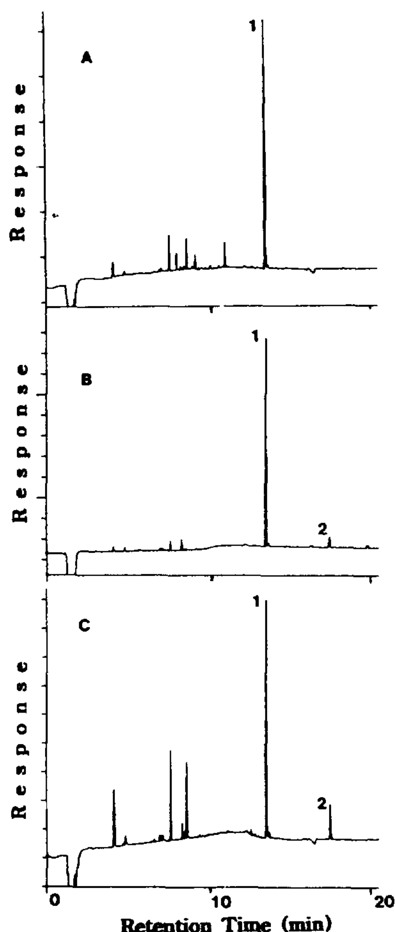

Fig. 2. Chromatograms of human plasma extracts. (A) Blank plasma; (B) plasma to which 200 ng/ml of verapamil were added; (C) plasma that is quantified as 396 ng/ml from a patient. Peaks: 1 = zipeprol (13.50 min); 2 = verapamil (17.53 min).

that the method is suitable for monitoring verapamil plasma levels during chronic administration of the drug. The data are reported in Table 3.

Table 2
Precision and accuracy of the determination of verapamil concentration in a 0.5-ml sample of human serum

Concentration added (μg/ml)	N	Concentration found (mean ± S.D.) (μg/ml)	C.V. (%)
0.100	3	0.099 ± 0.004	4.30
0.200	3	0.191 ± 0.004	2.26
0.500	3	0.491 ± 0.049	1.75

Table 3
Verapamil concentration in plasma at different time points by first injection (0.15 mg/kg) and by continuous i.v. infusion (0.2 mg/kg/h) with a daily dose escalation (of 0.05 mg/kg/h) of verapamil

Subject	Verapamil concentration (μg/ml)				
	Time after first injection (day)				
	1	2	3	4	5
GYS	0	0.344	0.426	0.770	0.668
KSN	0	0.691	0.812	0.283	0.260
YSM	0	0.107	0.165	0.579	0.884
CDJ	0	0.510	0.142	0.052	0.045
CBP	0	0.206	0.151	0.086	0.012
CMH	0	0.093	0.249	0.050	0.174
CGS	0	0.160	0.247	0.246	0.151
YJS	0	0.095	0.255	0.182	0.166
KMS	0	0.396	1.222	0.364	0.329
PCS	0	0.277	0.417	0.601	0.596
LSY	0	0.112	0.176	0.152	0.051
OMN	0	0.108	0.240	0.219	0.279
LHS	0	0.126	0.112	0.196	0.236
PJH	0	0.092	0.073	0.098	0.130

4. Discussion

To date, the HPLC methods have mainly been employed to determine verapamil concentration in biological samples, although GC–NPD has been widely used for measuring the trace levels of drugs. Our aim was to achieve not only high sensitivity, but also speed and simplicity. The method that uses HPLC is more time-consuming. The major features of NPD help overcome the problems of the quantitative assay. The high selectivity for nitrogen-containing compounds seems to provide a means for the further simplification of existing methods, by reducing the need for purification of the plasma extract prior to chromatography, thus reducing the time required for analysis. In this way, we reduced the time necessary to prepare twenty samples for GLC injection to less than 1 h. The fact that the cytotoxic agent adriamycin, associated with verapamil administration, does not interfere with the analysis is another advantage. The very good reproducibility of the method is due to the reliability of the NPD and to the minimal handling of samples.

The described assay confirmed the validity of the method for routine monitoring in cancer patients. Plasma concentrations found in patients treated with

verapamil are in agreement with those available in the literature.

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