

Rapid Determination Method of Caffeine and Application to Monitoring of Caffeine-assisted Chemotherapy

Masami Kawahara^a, Hiromi Kagiya^a, Yoshimitsu Kanazawa^b, Hiroyuki Tsuchiya^b, Katsuro Tomita^b, Koichi Yokogawa^a and Ken-ichi Miyamoto^{a,*}

^aDepartment of Pharmacy, Kanazawa University Hospital, Ishikawa, Japan

^bDepartment of Orthopaedic Surgery, School of Medicine, Kanazawa University 13-1, Takara-machi, Kanazawa, Ishikawa, 920-8641, Japan

ABSTRACT: Caffeine-assisted chemotherapy for bone and soft tissue tumours is very effective. However, high serum caffeine concentrations cause severe side effects, so monitoring of the serum level during therapy is important. For this purpose, a rapid determination method was established by high-performance liquid chromatography after solid-phase extraction. This method can measure caffeine and its three major metabolites in serum samples within 8 min. The mean serum caffeine concentrations of patients were 34.6 ± 7.8 , 54.5 ± 11.9 and $73.0 \pm 12.8 \mu\text{g/ml}$ at 24, 48 and 72 h, respectively, after the start of a $1500 \text{ mg/m}^2/\text{day}$ continuous infusion for 72 h. The distribution volume of caffeine was $0.65 \pm 0.23 \text{ l/kg}$, and the total body clearance was $0.025 \pm 0.011 \text{ l/h/kg}$, which was one-third of the reported low dose clearance. The appropriate infusion rate was calculated to avoid severe side effects in the final phase of the infusion by using a one-compartment constant infusion model based on the serum levels measured at 24 and 48 h. Caffeine clearance did not correlate with the metabolite/caffeine ratio in serum at any time. It is concluded that individual monitoring with this method for the purpose of dose adjustment is useful for avoiding the side effects of caffeine-assisted chemotherapy. Copyright © 2004 John Wiley & Sons, Ltd.

Key words: caffeine; chemotherapy; HPLC; osteosarcoma

Introduction

Bone and soft-tissue sarcomas frequently occur in young persons, although they are not common in adults. Chemotherapy, operation and radiotherapy are available as treatment options. Since the 1970s, xanthine derivatives including caffeine have been reported to inhibit DNA repair and to enhance the anti-tumour effect of cisplatin [1, 2]. Chemotherapy using caffeine together with traditional tumoricidal drugs has an excellent anti-tumour effect against bone and soft tissue tumours, and has been used at the Department of Orthopaedics, Kanazawa University, since 1989.

The combined chemotherapy with caffeine is effective against tumours which can not be treated with anticancer drugs alone. For example, the combination therapy is 100% effective against osteogenic sarcomas [3, 4]. According to the K2 protocol, $1500 \text{ mg/m}^2/\text{day}$ of caffeine is injected continuously for 72 h as shown in Figure 1. Care is necessary, since large doses of caffeine stimulate the central nervous system and can be fatal [5]. However, most pharmacokinetic studies of caffeine in humans have employed oral administration, and when caffeine was administered parenterally the maximum safe dose was only 30 mg/kg [6–8]. Furthermore, although the clinical application of caffeine-assisted chemotherapy has been reported [9–11], no information is available on the control of the caffeine concentration. In an *in vitro* study, the efficacy of the

*Correspondence to: 13-1, Takara-machi, Kanazawa, Ishikawa, 920-8641, Japan. E-mail: miyaken@pharmacy.m.kanazawa-u.ac.jp

eluate with 1 ml of methanol into the vessels. The eluted samples were evaporated to dryness under a stream of nitrogen gas, and each residue was dissolved in 500 μ l of the mobile phase.

HPLC analysis

The HPLC system was as follows, pump: LC-6A, system controller: SCL-6A, auto-injector: SIL-6A, column oven: CTO-6A, UV-detector: SPD-6A, recorder: C-R3 (Shimadzu Co., Kyoto, Japan). The analytical column was a Shimpack CLC-ODS, 6 mm \times 15 cm (Shimadzu Co., Kyoto, Japan).

The mobile phase was 0.1M NaH_2PO_4 in 30% methanol, the detection wavelength was 274 nm, and the flow rate was 1 ml/min, according to Tanaka [16]. 7-(β -hydroxypropyl)theophylline was selected for the internal standard according to the report of Deturmeny and Bruguerolle [17], because this subject is more stable and has a shorter retention time than 8-chlorotheophylline used in Tanaka's method. The injection volume was 20 μ l and the column temperature was set at 40°C.

Reproducibility

To examine the reproducibility of this method, the calibration samples of caffeine, theophylline, paraxanthine and theobromine were measured every day for 5 days.

Clinical application

The caffeine-assisted chemotherapy for bone and soft tissue tumours was planned as a clinical trial protocol in Kanazawa University Hospital. The study is in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of Kanazawa University Hospital. Patients were fully informed about the trial by their attending doctor, and gave written consent to participate. Blood sampling times were 24, 48, 72, 84 and 96 h from the beginning of caffeine administration. These sampling times were selected to avoid interfering with patients' sleep. Each blood sample was centrifuged for 5 min at 3000 rpm, then serum was collected and stored at -20°C until measurement. The distribution

volume, clearance and elimination rate constant were estimated by moment analysis (WinNonlin Standard, ver. 1.5, Pharsight Co., Mountain View, CA, USA).

Results

Establishment of simple and rapid determination method of caffeine

Figure 2 shows a representative chromatogram of a sample obtained from a patient. Caffeine, theophylline, paraxanthine, theobromine and the internal standard were well separated, without any interfering peaks. The required time was 20 min for sample preparation and 8 min for chromatographic analysis. The detection limits of this method were about 1.0 μ g/ml for caffeine and 0.2 μ g/ml for metabolites. Compared with Tanaka's method [16], this method showed excellent correlations between the observed and expected values in a set of 22 samples for caffeine: $y = 0.992x + 0.147$, $r^2 = 0.983$; theophylline: $y = 1.15x + 0.172$, $r^2 = 0.952$; paraxanthine: $y = 1.33x + 2.25$, $r^2 = 0.812$; theobromine: $y = 1.17x - 0.430$, $r^2 = 0.956$ (data not shown). Possible x values in this comparison were 0–100 μ g/ml for caffeine, and 0–20 μ g/ml for theophylline,

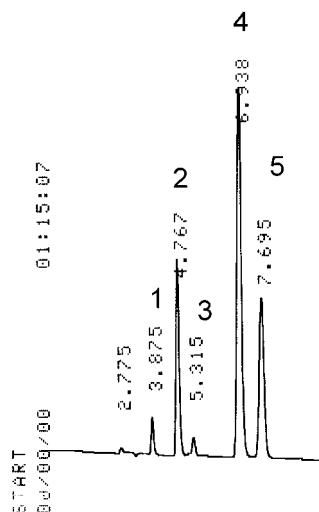


Figure 2. Chromatogram of caffeine and its metabolites in serum of a patient. 1: theobromine; 2: paraxanthine; 3: theophylline; 4: caffeine; 5: internal standard

paraxanthine and theobromine. The between-day precision of the method is shown in Table 1. The linear regression coefficients ranged from 0.997 to 1.000, and the coefficients of variation were within 10% in all samples.

Caffeine pharmacokinetics of patients at a dose of 1500 mg/m²/day for 72 h

Figure 3 shows the mean serum caffeine concentration-time profile for 35 patients who received a continuous intravenous infusion at a dose of 1500 mg/m²/day for 72 h without dose modification. The serum caffeine concentration rose throughout the administration period and did not reach a steady-state. The concentration peaked at the end of infusion, and then decreased promptly after the completion of administration. The mean serum caffeine concentrations of patients were 34.6 ± 7.8 µg/ml, 54.5 ± 11.9 µg/ml and 73.0 ± 12.8 µg/ml at 24, 48 and 72 h, respectively, after the start of the infusion. The pharmacokinetic parameters were calculated by moment analysis and the results are listed in Table 2. The parameters of healthy volunteers

given a dose of 5 mg/kg by intravenous infusion, reported by Blanchard and Sawers [18], are also listed in Table 2 for comparison. In our case, the distribution volume was similar to the reported value, but the clearance and elimination rate constant were only about one-third of the reported values.

Relationship between caffeine clearance and its metabolites

There was no significant relationship between the theophylline/caffeine, paraxanthine/caffeine and theobromine/caffeine ratios and the caffeine clearance at 24, 48 or 72 h after the start of caffeine infusion. Linear regression coefficients for these relationships were 0.201, 0.095, 0.115 for paraxanthine, 0.432, 0.002, 0.128 for theobromine, 0.200, 0.177, 0.138 for theophylline at 24, 48, 72 h after infusion, respectively ($n = 41$, data not shown).

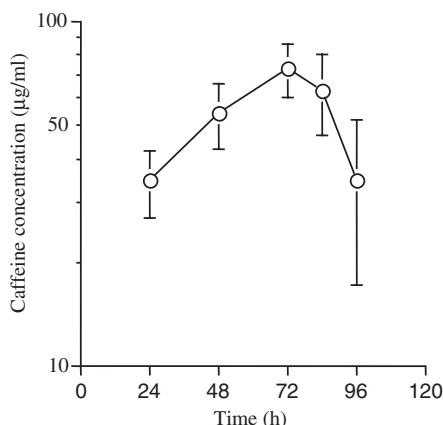


Figure 3. Caffeine concentration-time profile in patients. Patients were administered caffeine by constant infusion at a dose of 1500 mg/m²/day for 72 h. Each point with bar represents the mean ± SD ($n = 35$)

Table 1. Between-run precision for caffeine and its metabolites

Drug	Concentration (µg/ml)	Peak/IS Mean ± SD ($n = 6$)	CV (%)
Caffeine	12.5	1.02 ± 0.01	1.36
	25	1.75 ± 0.02	1.15
	50	3.16 ± 0.05	1.45
	100	6.01 ± 0.11	1.84
Theophylline	2.5	0.20 ± 0.01	3.58
	5	0.38 ± 0.01	3.22
	10	0.76 ± 0.03	4.01
	20	1.46 ± 0.05	3.11
Paraxanthine	2.5	0.41 ± 0.02	4.78
	5	0.61 ± 0.03	4.68
	10	1.01 ± 0.004	3.83
	20	1.76 ± 0.09	5.33
Theobromine	2.5	0.24 ± 0.01	4.77
	5	0.44 ± 0.004	1.00
	10	0.85 ± 0.007	0.77
	20	1.65 ± 0.02	1.20

CV coefficient of variation.

Table 2. Pharmacokinetic parameters of caffeine

	Cl (l/h/kg)	V _d (l/kg)	k _e (h ⁻¹)
This study ^a	0.025 ± 0.011	0.65 ± 0.23	0.044 ± 0.024
Blanchard and Sawers ^b	0.084 ± 0.030	0.61 ± 0.02	0.14 ± 0.050

Each value represents the mean ± SD.

^aIntravenous constant infusion at a dose of 1500 mg/m²/day for 72 h ($n = 35$).

^bIntravenous constant infusion for 30 min (total dose 5 mg/kg) cited from reference [18] ($n = 5$).

Clinical application

The side effects of caffeine, such as vomiting, sleeplessness and restlessness, appear at serum caffeine concentrations over 30 µg/ml, and become severe above 60 µg/ml. The side effects at concentrations under 60 µg/ml can be controlled sufficiently by the administration of appropriate anti-emetic drugs. The serum caffeine concentration decreased rapidly after completion of the infusion, and the side effects immediately disappeared.

The serum caffeine concentrations at 72 h were closely related to those at 48 h in 35 patients, as shown in Figure 4. The following equation was obtained.

$$C_{72h} = 0.797 \times C_{48h} + 29.3 \quad (1)$$

where C_{72h} is the caffeine concentration at 72 h and C_{48h} is the caffeine concentration at 48 h.

The correlation coefficient was 0.691 ($n = 59$), and the relationship was statistically significant ($p < 0.001$).

Relationship between caffeine pharmacokinetics and chemotherapy protocols

The histograms of caffeine clearance of 137 patients who received protocol 1 and 65 patients who received protocol 2 are shown in Figure 5. The caffeine clearance was 0.001–0.0391 l/h/kg in protocol 1, while it was 0.008–0.0671 l/h/kg in protocol 2, indicating large inter-individual variation. To minimize the effect of individual

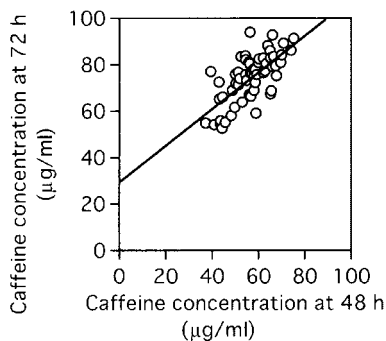


Figure 4. Correlation between caffeine concentrations at 48 h and 72 h after the start of intravenous infusion.

$$C_{72h} = 0.797 \times C_{48h} + 29.3 \quad (r = 0.691, p < 0.001)$$

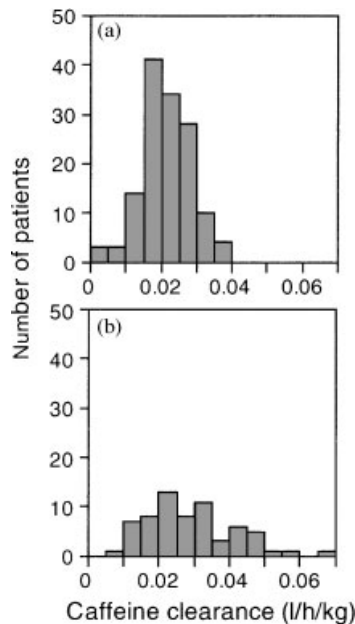


Figure 5. Distribution histograms of caffeine clearance in two protocols. (a) Protocol 1 ($n = 137$), (b) Protocol 2 ($n = 65$)

Table 3. Pharmacokinetic parameters of patients in the two protocols

	Cl (l/h/kg)	V_d (l/kg)	k_e (h^{-1})
Protocol 1	0.022 ± 0.006	0.66 ± 0.17	0.044 ± 0.048
Protocol 2	0.031 ± 0.012^a	0.51 ± 0.18	0.072 ± 0.045

Each value represents the mean \pm SD of 14 patients.

^aSignificantly different from protocol 1 at $p < 0.05$.

differences, 14 patients who received both protocols were selected and their pharmacokinetic parameters are listed in Table 3. The distribution volumes were almost the same, but the clearance in protocol 2 was significantly larger than that in protocol 1 ($p < 0.05$).

Discussion

Our HPLC determination method for caffeine and its primary metabolites is very rapid, easy and specific as shown in Figure 2. The extraction method by organic phase used in Tanaka's method was sometimes influenced by the extraction technique and condition, the calibration curves were required every measurement. The Oasis extraction column showed excellent reproducibility for caffeine monitoring, it was not

necessary to produce calibration curves for every measurement. With this advantage, the appropriate caffeine concentration could be calculated within about an hour, and in 65 of 221 patients the caffeine concentration was controlled safely. Furthermore, the sample volume was minimized to 50 μ l of serum. This was considered as a benefit for children.

Dichloromethane used in Tanaka's method [16] or tetrahydrofuran and acetonitrile used in Deturmeny's method [17] require proper disposal procedures rather than methanol. The control of the column temperature at a constant value of 40°C in our method improved the reproducibility of the retention times, which was important for routine monitoring. 7-(β -Hydroxypropyl)theophylline, used as an internal standard, was stable at 5°C in an aqueous stock solution for more than 6 months [17], and the calibration curves were highly reproducible.

Caffeine clearance is reported to be nonlinear in animals [19]. Cheng *et al.* reported dose-dependent pharmacokinetics of caffeine on oral administration in humans [20]. They reported that caffeine clearance decreased significantly when the oral caffeine dose was increased from 70 mg to 300 mg, while the distribution volume did not change. Furthermore, caffeine clearance in hepatic cirrhosis patients was decreased to one-fifth of that in normal subjects reflecting saturation of caffeine metabolism. Caffeine is metabolized predominantly by CYP1A2 [21], and its demethylation to paraxanthine, theophylline and theobromine is reported to be biphasic with high ($K_m < 0.33$ mM) and low-affinity ($K_m > 19$ mM) components [22]. Paraxanthine is the major primary metabolite of caffeine and its K_m value for the high-affinity component is 0.18 ± 0.02 mM [22]. The average serum caffeine concentration in patients at 24 h was 34.6 ± 7.8 μ g/ml, which is almost equal to a high-affinity K_m and metabolic saturation would have occurred at this massive dose.

Therefore, it was thought that the control of the caffeine concentration throughout the infusion would be important for safety reasons. As Landi *et al.* reported that there is wide intra and inter-individual variety in CYP1A2 activity [14], so it will be necessary to measure the clearances at several doses in some patients for the estimation of the Michaelis–Menten parameters. Patients

needed to receive the caffeine-assisted chemotherapy immediately, so the K_m and V_{max} of caffeine were not estimated in patients.

Based on equation (1), it was considered that patients whose caffeine concentration was over 63.6 μ g/ml at 48 h were likely to have an excessively high concentration at the end of the infusion. The caffeine concentration was estimated at 72 h from the concentrations at 24 and 48 h by using a one-compartment constant infusion model, and if necessary, the caffeine infusion rate was modified so that the final concentration would not exceed 80 μ g/ml. Sixty-five of 221 patients who received the caffeine-assisted chemotherapy had their caffeine infusion rate decreased on this basis, and as a result their serum caffeine concentration was controlled to below 80 μ g/ml at the end of the infusion period.

Caffeine is used as a probe drug for phenotyping of CYP1A2, and Fuhr *et al.* reported that the paraxanthine/caffeine ratio in saliva or plasma can be used to determine the CYP1A2 activity [23]. Since this method can simultaneously measure primary metabolites of caffeine, it can be employed to evaluate the metabolic capacity of each patient. However, the concentration of the three metabolites did not increase along with the caffeine concentration during the infusion period. No significant correlation was found of the metabolite/caffeine ratios in serum with caffeine clearance in this study (data not shown). Inter-individual differences in CYP1A2 activity are considered to be large, so it may be desirable to estimate the CYP1A2 activity prior to treatment of individual patients at a low dose where the relationship of the metabolite/caffeine ratio to clearance is still linear.

Furthermore, as shown in Figure 5, the caffeine clearance not only showed inter-individual variation, but also depended upon the chemotherapy protocol. Over 95% of caffeine is metabolized in the liver, and excretion of unchanged caffeine in urine amounts to only 5% or so [19]. Tang-Liu *et al.* reported that there was a significant correlation between caffeine clearance and the urine flow rate [6]. Sinues *et al.* also reported an influence of the urine flow rate on some caffeine metabolite ratios used to assess CYP1A2 activity, so control of liquid intake and urine flow are important [24]. The transfusion amount in pro-

TOCOL 2 was 0.6–1.01 more than in protocol 1, and diuresis by mannitol administration was done every 24 h for 3 days to avoid bladder damage by ifosfamide. The difference of caffeine clearance between these two protocols may be accounted for by these factors. It would be desirable to examine the relationship between the transfusion amount and the urine flow or creatinine clearance in patients receiving these protocols.

In conclusion, a rapid determination method of caffeine and its metabolites by HPLC was established. It was found that the pharmacokinetics of caffeine after administration of a massive dose is quite different from that after a low dose. This method is suitable for monitoring serum caffeine concentration in patients who are receiving caffeine-assisted chemotherapy, for the purpose of dose adjustment to prevent side effects.

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