—Regular Articles—

Clinical Estimation of Vancomycin Measurement Method on Hemodialysis Patient

Masayo TANAKA,*,[a] Takao ORII,[a] Tomoko GOMI,[b] Hiroyoshi KOBAYASHI,[c] Motoko KANKE,[d] and Shuichi HIRONO[e]

Department of Pharmacy,[a] Department of Nephrology,[b] and The Director of Hospital,[c] Kanto Medical Center NTT EC, Japan, 5–9–22, Higashigotanda, Shinagawa-ku, Tokyo 141–8625, Japan, Department of Clinical Pharmacy,[d] Kyoritsu College of Pharmacy, 1–5–30, Shibakoen, Minato-ku, Tokyo 105–8512 Japan, and Department of Pharmaceutical Sciences,[e] Kitasato University, 5–9–1, Shirokane, Minato-ku, Tokyo 108–8641, Japan

(Received September 11, 2001; Accepted January 22, 2002)

The fluorescence polarization immunoassay (FPIA) method is used to perform measurement of vancomycin hydrochloride (VCM) at many institutions. However, the values measured by the FPIA method are more vulnerable to overestimation than the high performance liquid chromatograpy (HPLC) method. In particular, it was not reported to perform exact therapeutic drug monitoring (TDM) measurement. Since overestimation is likely in patients with renal dysfunction, the HPLC method is preferable for TDM measurement. This study investigates the clinical conditions that lead to overestimation in the FPIA method, paying attention to the relation of clinical laboratory data inspection values and the existence of hemodialysis (HD). Overestimation in the evaluation of TDM using the FPIA method was clinically examined with 116 serum samples obtained from 18 cases medicated with VCM. The relevance between overestimation of patients who had not had HD performed was 72.7±61.7% (means±SD). In short, the overestimation was greatest in HD patients. Since overestimation did not affect the evaluation of clinical TDM, such as an effect and a side-effect, the TDM of VCM was shown to be satisfactorily evaluated by the simple FPIA method.

Key words——vancomycin; HPLC; FPIA; hemodialysis; overestimation factors

INTRODUCTION

VCM is an effective drug for the treatment of infections caused by methicillin-resistant-staphylococcus aureus (MRSA); however, TDM is a necessary part of this treatment since VCM is nephrotoxic and ototoxic. Presently, at many institutions, TDM for VCM is performed immunologically using polyclonal antibodies (fluorescence polarization immunoassay: FPIA method) (TDX, Dynabbott Inc.). However, it has been reported that when the concentration of VCM was determined by this method, VCM of a crystalline degradation product 1(CDP–1) cross-reacts with VCM. CDP–1 is the degradation of VCM and has no antimicrobial activity. This CDP–1 leads to an overestimation. And it has been that correct TDM is not performed because the measurement data by FPIA method is more overestimation than the measurement data by HPLC method.[1,2] Since the excretion of VCM takes a significantly longer time in patients with nephropathy, CDP–1 is more likely to be produced. Hence, the concentration of VCM could easily be overestimated by the FPIA method in these patients.[3—6] To date, no reports have described the influence of the overestimation of VCM on hemodialysis (HD), clinical laboratory test results, or the effectiveness or side-effects of infection therapy.

Hence, the present study investigated (1) differences in VCM concentrations determined by the FPIA and HPLC methods, (2) the correlation between overestimation and the effectiveness and side-effects of therapy, (3) the correlation between overestimation and the patient being on HD.

MATERIALS AND METHODS

1. Subjects We examined a total of 116 serum samples collected from 18 patients who received VCM between May 1, 1998 and January 31, 1999 at the Kanto Medical Center NTT EC, Japan. Table 1 shows patient profiles and the number of samples collected from each patient.

2. Reagents and Measurement methods Standard VCM ($C_{66}H_{75}Cl_2N_9O_{24}$) for research and experiments (manufacturing code: VM16662, donated by Shionogi & Co., Ltd.) were used. Human Serum R (Biowhittaker: lot no. 7M0638) was used as standard human serum, and ristocetin (Sigma Chemical Co., Ltd.) was used as the internal standard. The other

e-mail: tanaka@kmc.mhc.east.ntt.co.jp

Table 1. Characteristics of Patients

Patient No.	Age (Year)	Sex	Number of samples	HD	Primary diseases	VCM use infection
1	65	M	30	+	Sternal myelitis, aortic regurgitation	sepsis
2	70	M	4	−	Acute suppurative cholangitis, acute pancreatitis, CBD stone	sepsis
3	52	M	3	−	Gastric carcinoma	sepsis
4	93	F	2	−	Gastrointestinal bleeding	sepsis
5	78	F	2	−	Disturbance of consciousness, cerebral embolism	sepsis
6	53	M	2	−	Acute lymphocytic leukemia	MRSA pneumoniae
7	86	F	1	−	Parkinson's disease	sepsis
8	68	F	2	−	Acute lymphocytic leukemia	sepsis
9	72	M	6	−	Upper gastrointestinal bleeding, lleus	MRSA pneumoniae
10	80	F	1	−	Chronic inflammatory demyelinating polyneuropathy	sepsis
11	60	M	1	−	Cerebral infarction	sepsis
12	62	M	5	+	Lumbardisc discopathy, Infective endocarditis, tricuspid insufficiency	sepsis
13	53	M	8	−	Esophagus carcinoma	MRSA pneumoniae
14	59	F	15	−	Acute pancretitis	MRSA spomdykitis
15	62	M	12	+	Acute myocardial infarction	sepsis
16	65	M	2	−	Gastric carcinoma	MRSA pneumoniae
17	78	M	2	+	MRSA pneumoniae, nephrosclerosis	MRSA rneumoniae
18	20	M	18	−	Dorsolateral abscess	MRSA spomdykitis

HD: hemodialysis.

chemicals used were all reagent grade.

The serum concentrations of VCM were measured by HPLC according to the method of Murayama et al.[5] The serum concentration of VCM was measured by FPIA automatically using an Abbot's TDX.

3. Preparation of a Calibration Curve Standard VCM was dissolved in standard human serum to produce 1, 5, 20, 40 and 60 μg/ml solutions, and a calibration curve was prepared by measuring the VCM concentration of each solution three times.

The HPLC standard curve was made from the concentration and the height of peak value. The HPLC standard curve was $Y=334.2X$ ($R^2=0.9998$). The VCM concentration of samples were calculated with the standard curve.

4. Diurnal and Daily Fluctuation, and the Estimation of FPIA Calibration VCM was dissolved in standard human serum to produce 2.5, 10, 30 and 50 μg/ml solutions, and the VCM concentration of each solution was measured five times of the day.

VCM was dissolved in standard human serum to produce 2.5, 10 and 30 μg/ml solutions, and the VCM concentration of each solution was measured on six different days.

At each concentration, the coefficient variation (CV) was less than 1% for the HPLC method, while it was less than 2.5% for the FPIA method. The CV was favorable with the HPLC method, except when it reached 2.08% at the VCM concentration of 10 μg/ml and 1.17% at 30 μg/ml. With the FPIA method, the CV was also favorable, except when it reached 2.38% at 10 μg/ml and 2.54% at 30 μg/ml.

The estimation of the FPIA calibration stability was done with two lots. The concentrations of the solution VCM was dissolved 4.7, 15.4, 29.7 and 54.1 μg/ml were measured by the calibration of two lots.

The calibration stability of TDX were 5.5% at 4.7 μg/ml, 1.7% at 15.4 μg/ml, 1.4% at 29.7 μg/ml and 54.1 μg/ml with lot 1, and 5.6% at 4.7 μg/ml, 1.7% at 15.4, 1.4% at 29.7 μg/ml and 54.1 μg/ml with

other lot. And when it was low concentration, there was a tendency for a certain degree CV to be large.

5. Measurements Using Patient Serum Samples According to the above-mentioned procedure, each serum sample that was stored at −20℃[6] was subjected to HPLC three times. Then, using the calibration curve and the chromatogram, the concentration of VCM was calculated.

On the day of blood sampling, serum was isolated by centrifugation and subjected to analysis using TDX.

6. Calculation of Differences in the Measured Drug Concentrations (Overestimation) between the FPIA and HPLC Methods The difference in the measured drug concentrations between the FPIA and HPLC methods was calculated based on actual measurements using the following formula:

Degree of overestimation (%)
= [(FPIA value-HPLC value)/HPLC value] × 100

7. Test Items Clinical laboratory data was used as the data of VCM measurement data of the day.

Clinical laboratory tests for total bilirubin (T-bil), glutamyl oxaloacetic transaminase (GOT), glutamyl pyruvic transaminase (GPT), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (γ-GTP), lactate dehydrogenase (LDH), blood urea nitrogen (BUN) and serum creatine (Cr) levels were conducted, and the conditions of infection were assessed by bacterial culture, C-reactive protein (CRP) levels, white blood cell (WBC) counts and body temperature. In addition, various patient background factors, including age, body weight, present illness, past medical history, nephropathy, hepatopathy, duration of VCM administration, doses and coadministered drugs, were analyzed. The analysis of the pharmacokinetics parameter was done with two point of the value after 2 hours from administration and the trough value, these two values are the study state.

Furthermore, using the concentration of VCM measured by the FPIA method, the initial values of the kinetic parameters of VCM were calculated by the Sawchuk-Zaske method. Then, half-life and distribution volume were calculated using Bayesian analysis software (Higuchi, Kyushu University, Japan).

The clinical effect was evaluated with CRP, WBC and body temperature.

The onset of side-effects, such as hearing impairment, red neck syndrome, hepatic and renal dysfunctions was studied.

Table 2. Correlation between Overestimation and Laboratory Data ($n=116$)

Laboratory test item	Correlation coefficient (γ)	Laboratory test item	Correlation coefficient (γ)
T-Bil	0.0921	LDH	0.0869
GOT	0.0555	BUN	0.0126
GPT	0.08	Cr	0.0008
ALP	0.0286	K	0.0709
γ-GTP	0.0693		

8. Analysis items The correlation between the drug concentration as measured by the FPIA and HPLC methods was assessed using regression analysis of all 116 samples. The correlation between overestimation and various clinical laboratory test results was then evaluated using regression analysis.

The relationships between patient background factors and effectiveness and side-effects of therapy and between overestimation was analyzed for each patient. The clinical condition of the patient was assessed as "Significantly improved" when the levels of WBC count, CRP level and body temperature decreased and the elimination of MRSA was confirmed; "Improved" when the three laboratory data showed improvement levels, even though the elimination of MRSA was not confirmed; or "Unchanged" when the three laboratory data did not show any improvement and MRSA was still detected. Hepatic and renal functions at the beginning of and following VCM administration were evaluated according to the side-effects grading system proposed by the Ministry of Health , Labour and Welfare of Japan.[7] More than one grade might indicate the possibility of onset of a side-effect.

RESULTS

1. The Differences between FPIA and HPLC Methods

a. VCM Concentrations in Standard Serum Solutions To ascertain the degree of overestimation, standard VCM was dissolved in standard human serum to produce 1, 5, 20, 40 and 60 μg/ml solutions. Although the serum concentrations of VCM determined by the FPIA method were higher than those determined by the HPLC method at all concentrations, the degree of overestimation was less than 30 %. The tendency was that the lower the VCM concen-

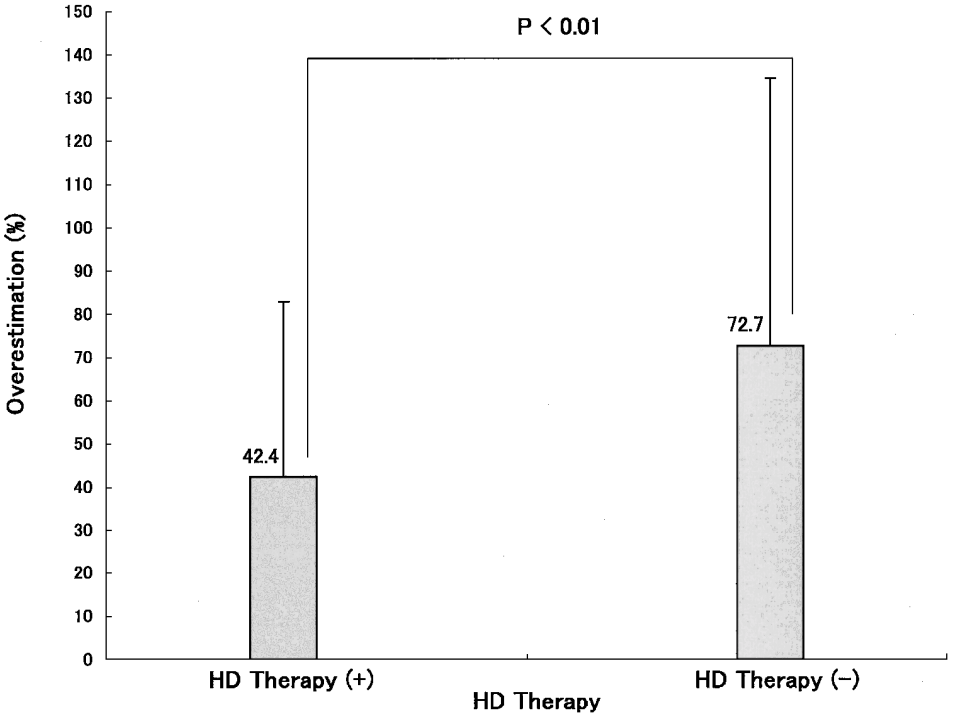

Fig. 1. Correlation of Overestimation and HD Therapy
mean ± SD (n=49).

Table 3. Clinical Datas and Pharmacokinetic Parameters

Patient number	Liver dysfunction	Renal dysfunction	$T_{1/2}$ (hr)	Vd (l/Kg)	Efficacy	Side-effect	Overestimation (%) Trough	C_{max}	all data
1	##	##	20.73	1.60	±	−	35.63	27.81	32.6 ± 30.4
2	##	−	not calculate		−	−	47.95	−	47.1 ± 2.7
3	##	−	10.68	1.00	+	−	63.85	67.08	64.9 ± 8.1
4	−	−	18.14	0.99	+	−	45.27	44.88	45.1 ± 0.3
5	−	−	13.41	0.86	±	−	34.28	44.52	39.4 ± 7.2
6	+	−	9.11	1.01	±	−	131.48	41.71	86.6 ± 63.5
7	−	−	5.47	0.44	+	−	60.49	−	60.5
8	−	−	11.23	0.91	±	−	46.38	32.66	39.5 ± 9.7
9	+	−	10.63	0.91	+	−	51.87	56.92	63.5 ± 7.2
10	+	−	12.85	0.72	+	−	−	52.80	52.8
11	−	−	not calculate		+	−	−	23.24	23.2
12	+	##	43.00	1.64	±	−	17.24	11.60	18.5 ± 7.3
13	−	+	3.03	0.99	+	−	204.86	83.93	126.9 ± 83.9
14	+	−	7.42	0.51	±	−	41.36	58.27	52.8 ± 13.5
15	+	+	61.98	1.15	+	−	69.33	78.15	77.7 ± 53.5
16	###	−	not calculate		+	−	6.93	−	6.9 ± 5.1
17	+	###	20.34	0.77	−	−	6.77	30.46	18.6 ± 16.8
18	+	−	6.50	0.87	+	−	49.07	43.32	74.7 ± 69.3

Patient No. 1, 12, 15 and 17 were on HD therapy.
Efficacy: + Remarkably improved, ± Improved, − Unchanged
Liver dysfunction: Classified by the grade proposed by the Ministry of Health, Labour and Welfare of Japan.
Renal dysfunction: − Cr < 2.0 g/dl, + 2.0 g/dl ≦ Cr < 4.0 g/dl, ## Cr ≧ 4.0 g/dl
All data (mean ± SD) was calculated by being together all measurement data regardless of trough value or peak value.

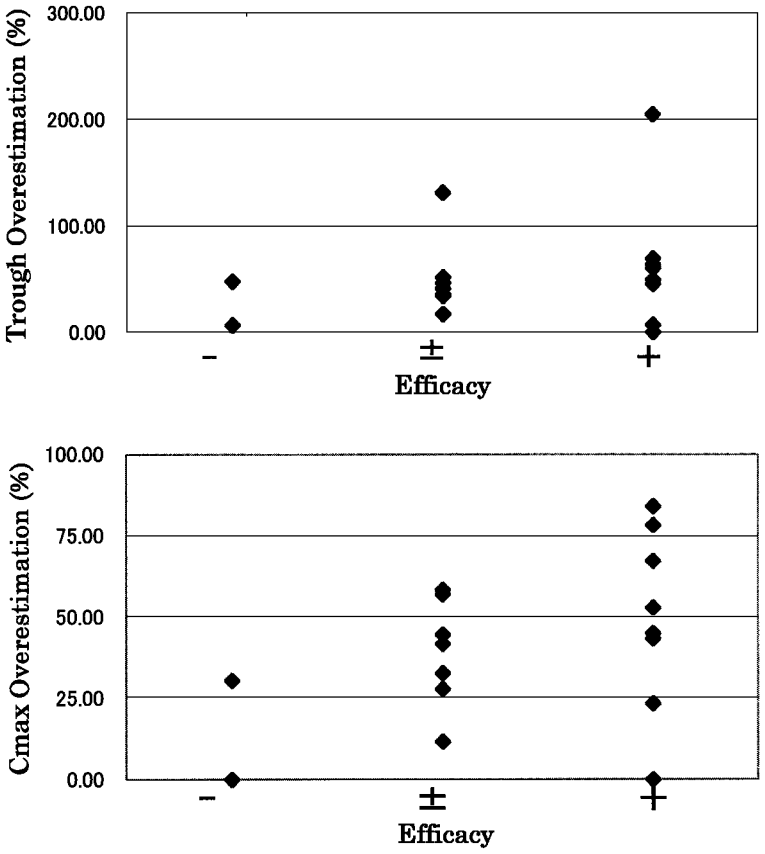

Fig. 2. Correlation of the Traugh Overestimation and Clinical Efficacy and Correlation of the Cmax Overestimation and Clinical Efficacy
− Unchanged, ± Improved, + Remarkably improved.

tration, the higher the degree of overestimation.

b. Measurements of Patient Serum Samples Except for 2 of the 116 samples, the concentrations of VCM measured by the FPIA method were higher than those measured by the HPLC method. The degree of overestimation ranged from −21.4% to 306.3%.

2. Correlation between the Degree of Overestimation and Laboratory Test Results and the Pathology

The relationship between the degree of overestimation and each set of clinical laboratory test results was assessed by regression analysis. No significant correlations were observed between the degree of overestimation and the hepatic and renal functions, or other clinical laboratory test results (Table 2).

Four patients were on HD, and 49 samples were collected from these patients. The degree of overestimation was 42.4% (40.5% in the samples obtained from the patients on HD and 72.7% (61.7% in the remaining samples (68 samples from 14 patients who were not on HD); thus, the degree of overestimation was significantly smaller in the samples obtained from the patients on HD ($p<0.01$: $p=0.007$) (Fig. 1). Table 3 and Fig. 2 shows the degree of overestimation, kinetic parameters and the effectiveness and side-effects of therapy for each patient. However, there were no correlations between overestimation and kinetic parameters, or effectiveness and side-effects of therapy.

To reduce its nephrotoxicity, the trough concentration of VCM is generally set below 10 μg/ml. Therefore, the degree of overestimation was compared between samples containing VCM at concentrations above and below this level. The results showed a significant difference between the samples of VCM concentration less and those more than 10 μg/ml (80.5% (82.1% and 49.0% (31.0%, respectively) ($p=0.0038$) (Fig. 3). The tendency was that the lower the VCM concentration, the greater the overestimation. There have been no reports that the overestimation is estimated with over than 10 μg/ml or less than 10 μg/ml of FPIA method measurement value. It is reported that instability of calibrator is increased at low concentration. The same results were obtained by this

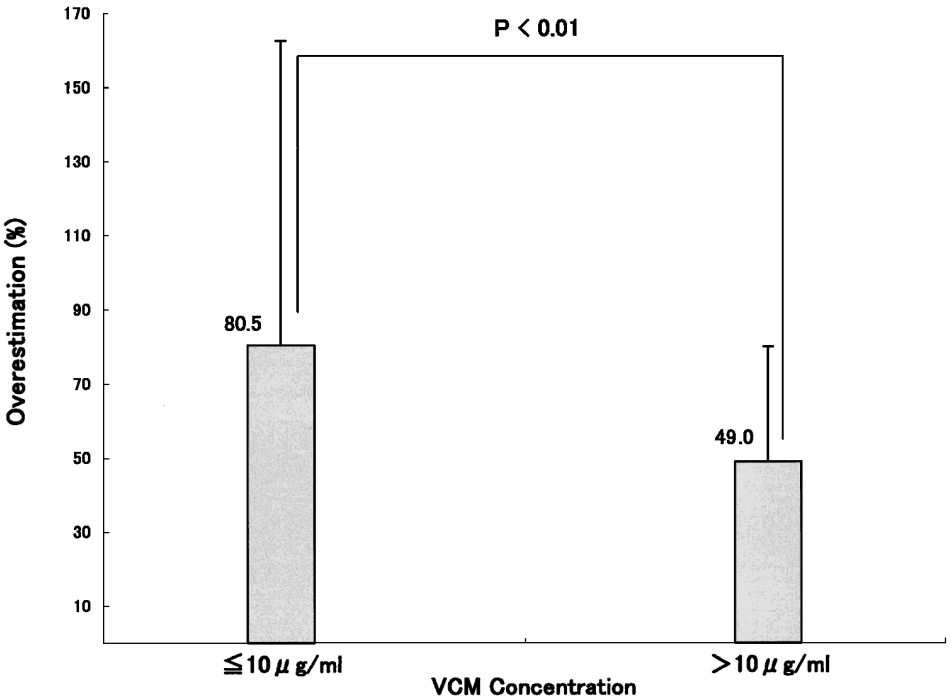

Fig. 3. Correlation of VCM Concentration and Overestimation
mean±SD

study. Morishige[8] comes to the conclusion that the cause of overestimation is to be produced CDP−1, but it did not come to the conclusion that the instability of calibrator is due to CDP−1 because the measurement of CDP−1 was not done in this study.

DISCUSSION

Patients with renal dysfunction produce CDP−1 by retaining VCM in their body for long time; as a result, it has been reported[1,2] that overestimation occurs. There was no correlation between overestimation and half-life.

Hence, the fact that the degree of overestimation was smaller for the patients on HD suggests that TDM can be performed more accurately for patients on HD.

A study on the stability of FPIA calibrators by Morishige et al.[8] reported that, after 30 days of storage at 4℃ and 25℃, VCM potency became lower by 20% and 60%, respectively. It has also been reported that the production of CDP−1 made these FPIA calibrators unstable, thus making overestimation more likely. Moreover, in this study, when concentration of VCM 10 μg/ml or less were compared with concentrations over 10 μg/ml, overestimation occurred more frequently with concentrations of 10 μg/ml or less ($p=0.0038$). The present study revealed that the greater the overestimations, the lower the concentrations of VCM, thus suggesting that the most significant factor in overestimation was the instability of FPIA calibrators. Therefore, the stability of calibrators is required in order to prevent overestimation. It can be assumed, that if TDM is performed using the FPIA method, the trough level may be raised due to overestimation, which reduces the risk of inducing nephropathy, but on the other hand, lowers the therapeutic value. However, the results of the present study did not show any correlation between kinetic parameters and overestimation and between effectiveness and side effects of therapy. Hence, we believe that overestimation is not a clinically significant issue.

Furthermore, overestimation of VCM bears no relation to treatment in terms of effectiveness and side effects. Moreover, it is unthinkable that the TDM that was used as overestimation data creates problems clinically. Therefore, the TDM of VCM can be carried out by the FPIA method without any problems, even in patients with nephropathy or HD.

REFERENCES

1) Morse G. D., Nairn D. K., Bertino J. S ., Wal-

she J. J., *Ther. Drug Monit.*, **9**, 212–215 (1987).
2) Paap C. M., Sharpe G. L., *Dev. Pharmcol. Ther.*, **20**, 174–179 (1993).
3) Najjar T. A., al-Dhuwailie A. A., Tekle A., *J. Chromatogr., Biomed. B.*, **672**, 295–299 (1995).
4) Follin S. L., Mueller B. A., Scott M. K., Carfagna M. A., Kraus M. A., *Am. J. Kidney Dis.*, **27**, 67–74 (1996).
5) Murayama T., Shibata M., Watanabe A., Yokoyama Y., Matsuda S., *Jpn. J. TDM.*, **13**, 30–35 (1996).
6) Mollering. R. C Jr., Krogstad D. J., Greenblatt D. J., *Ann. Intern. Med.*, **94**, 343–346 (1981).
7) International Medical Center of Japan, *Drug Information.*, **19**, 792–813 (1992).
8) Morishige H., Shuto H., Ieiri I., Otsubo K., Oishi R., *Ther. Drug Monit.*, **18**, 80–85 (1996).