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Effects of Cysteine on the Pharmacokinetics of Itraconazole in Rats with Protein-Calorie Malnutrition

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ABSTRACT: The effects of cysteine on the pharmacokinetics of itraconazole were investigated after intravenous, 20 mg/kg, and oral, 50 mg/kg, administration of the drug to control rats (fed for 4 weeks on 23% casein diet) and rats with PCM (protein-calorie malnutrition, fed for 4 weeks on 5% casein diet) and PCMC (PCM with oral cysteine supplementation, 250 mg/kg, twice daily during the fourth week). After intravenous administration of itraconazole to rats with PCM, the area under the plasma concentration–time curve from time zero to time infinity (AUC) of itraconazole was significantly greater (3580 compared with 2670 and 2980 μg min/ml) than those in control rats and rats with PCMC (the values between control rats and rats with PCMC were not significantly different). The above data suggested that metabolism of itraconazole decreased significantly in rats with PCM due to suppression of hepatic microsomal cytochrome P450 (CYP) 3A23 in the rats. The results could be expected since in rats with PCM, the level of CYP3A23 decreased significantly as compared to control. Itraconazole was reported to be metabolized via CYP3A4 to several metabolites, including hydroxyitraconazole, in human subjects. Human CYP3A4 and rat CYP3A1 (CYP3A23) proteins have 73% homology. By cysteine supplementation (rats with PCMC), the AUC of itraconazole was restored fully to control levels.

Key words: itraconazole; pharmacokinetics; PCM; cysteine; CYP3A23; rats

Introduction

Itraconazole, (±)-*cis*-4-[4-[4-[4-[[2-(2,4-dichlorophenyl)-2(1*H*-1,2,4-triazol-1-yl-methyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]-2,4-dihydro-2(1-methyl-propyl)-3*H*-1,2,4-triazol-3-one, is a prototype of triazole antifungal agent. The terminal half-life ($t_{1/2}$), protein binding, apparent volume of distribution, time-averaged total body clearance (Cl), urinary excretion and extent of absolute oral bioavailability (*F*) of itraconazole in humans were 21 ± 6 h, 99.8%, 14 ± 5 l/kg, 23 ± 10 ml/min/kg, less than 1% and 55%, respectively [1]. The terminal $t_{1/2}$, Cl and apparent volume of distribution at steady state (V_{ss}) of itraconazole were 4.9 ± 5.6 h, 14.2 ± 7.6 ml/min/kg and 6.0 ± 2.5 l/kg, respectively, after intravenous administration of the drug to 4 rats [2].

The following results were reported from our laboratories [3]. Western and Northern blot analyses revealed that in rats with protein–calorie malnutrition (PCM, 5% casein diet for 4 weeks), the protein and mRNA levels of the hepatic microsomal cytochrome P450(CYP) 1A2, CYP2E1, CYP2C11 and CYP3A23 decreased compared with control (23% casein diet for 4 weeks). Interestingly, the altered cytochrome

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P450 expression by PCM completely or partially returned to the level of control by oral cysteine supplementation for one week (250 mg/kg, twice daily during the fourth week, PCMC). Hence, in rats with PCMC, some pharmacokinetic parameters of drugs which were mainly metabolized by CYP1A (azosemide), CYP3A (adriamycin) or CYP2E1 (chlorzoxazone) were retuned to control [4–6]. The effects of cysteine to counteract changes in some pharmacokinetics of drugs due to PCM was proven for azosemide [4], adriamycin [5] and chlorozoxazone [6]. It was also reported [7, 8] that CYP3A4 is involved in the metabolism of itraconazole to form several metabolites, including hydroxyitraconazole, the major metabolite, in human subjects. Human CYP3A4 and rat CYP3A1 (CYP3A23) proteins have 73% homology [9]. Therefore, it could be expected that the metabolism of itraconazole decreased in rats with PCM and returned to control in rats with PCMC.

PCM as well as many selective mineral and vitamin deficiencies deteriorate the immune and inflammatory metabolic response, increasing the frequency of infections and worsening their evolution and prognosis [10]. Patients with cell-mediated immune dysfunction such as AIDS are susceptible to mucotaneous candidiasis and pulmonary and disseminated cryptococcosis [11]. The patients with PCM suffering from fungal infections could use itraconazole. Hence, the itraconazole was chosen in the present study. The purpose of this paper is to report the pharmacokinetic changes of itraconazole after intravenous, 20 mg/kg, and oral, 50 mg/kg, administration of the drug to control rats and rats with PCM and PCMC. The use of cysteine to counteract changes in pharmcokinetics of itraconazole due to PCM was also reported.

Materials and methods

Chemicals

Sporanox® intravenous solution (10 mg/ml as itraconazole, Lot No. 55-051-DH), Sporanox® oral solution (10 mg/ml as itraconazole, Lot No. 00GB404) and the powder of itraconazole and R51012 [the internal standard for high-performance liquid chromatographic (HPLC) assay] were supplied by Janssen Korea (Seoul, Republic of Korea). Other chemicals were of reagent grade or HPLC grade, and therefore were used without further purification.

Rats and diets

Male Sprague–Dawley rats, weighing 150–180 g, were purchased from Charles River Company (Atsugi, Japan). Rats were assigned randomly to one of the two diets containing either 23% (control rats) or 5% (rats with PCM) casein. Both diets were isocaloric and the compositions of the diets were listed [3]. All rats were provided with food and water ad libitum and maintained on each diet for a 4-week period (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University, Seoul, Republic of Korea). From the start of the fourth week, rats with PCM were divided randomly into two groups. One group was treated with 250 mg/kg of oral cysteine twice daily (cysteine was dissolved in tap water to make 100 mg/ml, rats with PCMC) and other group (without cysteine) was treated with the same volume of tap water (rats with PCM).

Pretreatment of rats

In the early morning after a 4-week feed on each diet, the jugular vein (only for intravenous study for drug administration) and the carotid artery (for blood sampling) of each rat were catheterized with polyethylene tubing (Clay Adams, Parsippany, NJ) under light ether anaesthesia. Both cannulas were exteriorized to the dorsal side of the neck where each cannula terminated with a long Silastic tubing (Dow Corning, Midland, MI). Both Silastic tubings were inserted into a wire to allow free movement of the rats. After the exposed areas were surgically sutured, each rat was housed individually in a rat metabolic cage (Daejong Scientific Company, Seoul, Republic of Korea) and allowed to recover from the anaesthesia for 240–300 min before the study began. They were not restrained during the whole experimental period.

Intravenous study

Itraconazole (Sporanox® intravenous solution), 20 mg/kg, was administered over 1-min via the jugular vein of control rats ($n = 10$) and rats with PCM ($n = 7$) and PCMC ($n = 10$). The injection volume was 0.6 ml/300 g. Approximately 0.12-ml aliquot of blood was collected via the carotid artery at 0 (to serve as a control), 1 (at the end of the infusion), 5, 15, 30, 45, 60, 120, 180, 240, 360, 540, 720, 1440, 2160 and 2880 min after intravenous dosing and a 50-μl aliquot of each plasma sample was stored in a −70°C freezer until HPLC analysis of itraconazole [12]. Approximately 0.3-ml aliquot of heparinized 0.9% NaCl-injectable solution (20 U/ml) was used to flush the cannula to prevent blood clotting after each blood sampling. Urine samples were collected between 0–1440 and 1440–2880 min. At each urine collection time, the metabolic cage was rinsed with 15 ml of distilled water and the rinsings were combined with each urine sample. After measuring the exact volume of each urine output and combined urine, aliquots of each urine samples were stored in a −70°C freezer until the analysis of itraconazole [12]. At the end of 2880 min, as much blood as possible was collected via the carotid artery and each rat was sacrificed by cervical dislocation. At the same time, the entire gastrointestinal tract (including its contents and faeces) was removed, transferred into a beaker containing 100 ml of methanol (to facilitate the extraction of intraconazole) and cut into small pieces using scissors. After stirring with a glass rod, two 50-μl aliquots of the supernatant were collected from each beaker and stored in a −70°C freezer until HPLC analysis of itraconazole [12].

Oral study

Itraconazole (Sporanox® oral solution), 50 mg/kg, was administered orally to control rats ($n = 8$) and rats with PCM ($n = 12$) and PCMC ($n = 7$) using a feeding tubing. The oral volume was 1.5 ml/300 g. The blood sampling times were 0 (to serve as a control), 30, 60, 120, 180, 240, 360, 540, 720, 1440, 2160 and 2880 min after oral administration of the drug. The other procedures were similar to those of intravenous study.

HPLC analysis of itraconazole

The concentrations of itraconazole in the above samples were determined by a slight modification of the reported HPLC method [12]. In a 2.2-ml eppendorf tube containing a 50-μl aliquot of plasma sample, a 50-μl aliquot of internal standard (0.2 μg/ml dissolved in acetonitrile) and a 250-μl aliquot of 0.1 M carbonate buffer (pH = 10) were added. After vortex-mixing for 30 s, the mixture was extracted with 1 ml of tert-butyl methyl ether. The organic layer was evaporated under nitrogen gas at 65°C. The residue was then reconstituted with a 100-μl aliquot of the mobile phase and a 75-μl aliquot was injected directly onto the HPLC column. The mobile phase, 20 mM KH_2PO_4 (pH = 2) : acetonitrile : 85% phosphoric acid = 50 : 50 : 0.15 (v/v/v), was run at a flow rate of 2.0 ml/min and the column effluent was monitored by a fluorescence detector set at an excitation wavelength of 260 nm and an emission wavelength of 364 nm. The retention times for itraconazole and the internal standard were approximately 8.2 and 12.7 min, respectively. The detection limit for itraconazole in plasma was 50 ng/ml. The coefficients of variation of the assay were generally low, below 7.52%. No interferences from endogenous substances were found.

The HPLC system consisted of an AS-1559 autosampler (Jusco, Tokyo, Japan), a model P-580 pump (Dionex, München, Germany), a reversed-phase (C_{18}) inertsil ODS-3 V column (4.6 mm, i.d. × 150 mm, *l*; particle size, 5 μm; GL Sciences, Tokyo, Japan), a model FL 3000 fluorescence detector (Thermo Separation Products, Riviera Beach, FL) and a model chromatocorder 21 integrator (System Instruments, Santa Fe, CA).

Pharmacokinetic analysis

The total area under the plasma concentration–time curve from time zero to time infinity (AUC) or up to the last measured time, 2880 min, in plasma ($AUC_{0–2880\,min}$) was calculated by the trapezoidal rule method; this method utilized the logarithmic trapezoidal rule [13] for the calculation of the area during the declining plasma–level phase and the linear trapezoidal rule for the rising plasma–level phase. The area from the last data point to time infinity (for the calculation of

AUC) was estimated by dividing the last measured plasma concentration by the terminal rate constant.

Standard methods [14] were used to calculate the following pharmacokinetic parameters after intravenous administration: the Cl, area under the first moment of plasma concentration–time curve (AUMC), mean residence time (MRT), V_{ss} and time-averaged renal (Cl_r) and nonrenal (Cl_{nr}) clearances [15].

$$Cl = dose/AUC \quad (1)$$

$$AUMC = \int_0^\infty t\,C_p\,dt \quad (2)$$

$$MRT = AUMC/AUC \quad (3)$$

$$V_{SS} = Cl\,MRT \quad (4)$$

$$Cl_r = A_{e0-2880\,min}/AUC \quad (5)$$

$$Cl_{nr} = Cl - Cl_r \quad (6)$$

where C_p is the plasma concentration of itraconazole at time t, and $A_{e\ 0–2880\,min}$ is the total amount of unchanged itraconazole excreted in urine for up to 2880 min. The Cl_r after oral administration was calculated by dividing $A_{e\ 0–2880\,min}$ by $AUC_{0–2880\,min}$.

For comparison, the F was estimated by dividing $AUC_{0–2880\,min}$ after oral administration by AUC after intravenous administration with dose normalization. Hence, the F value could be somewhat underestimated.

The mean values of each clearance [16], V_{ss} [17] and terminal half-life [18] were calculated by the harmonic mean method.

Statistical analysis

A $p<0.05$ was considered to be statistically significant using a Duncan's multiple range test of statistical package for the social sciences (SPSS) *posteriori* analysis of variance (ANOVA) program among the three means for unpaired data. All results are expressed as mean $\pm$ S.D.

Results

Pharmacokinetics after intravenous administration of itraconazole

The mean arterial plasma concentration–time profiles of itraconazole after intravenous administration of the drug, 20 mg/kg, to control rats ($n = 10$) and rats with PCM ($n = 7$) and PCMC ($n = 10$) are shown in Figure 1, and relevant pharmacokinetic parameters are listed in Table 1. After intravenous administration of itraconazole, the plasma concentrations declined in a polyexponential fashion for all three groups of rats (Figure 1) with mean terminal half-lives of 865, 1280 and 1160 min for control rats and rats with PCM and PCMC, respectively (Table 1). The half-lives were not significantly different among three groups of rats mainly due to considerable intersubject variations; the coefficients of variation were 43.8, 84.4 and 68.9% for control rats and rats with PCM and PCMC, respectively (Table 1). The MRTs were also not significantly different among three groups of rats (Table 1). The plasma concentrations of itraconazole in rats with PCM were higher than those in control rats and rats with PCMC (Figure 1). As a result, the AUC of itraconazole in rats with PCM was significantly greater than those in control rats and rats with PCMC; the value in rats with PCM was 34.1 and 20.1% greater than those in control rats and rats with PCMC, respectively (Table 1). However, the AUCs of itraconazole were not significantly different between control rats and rats with PCMC (Table 1). The significantly greater AUC of itraconazole in rats with PCM could be due to significantly slower Cl of itraconazole (25.4% decrease) than that in control rats (Table 1). The slower Cl in rats with PCM was due to significantly slower Cl_{nr} of itraconazole since Cl_r values were not significantly different between two groups of rats; the Cl_{nr} in rats with PCM was significantly slower (26.0% decrease) than that in control rats (Table 1). In rats with PCMC, the Cl_r of itraconazole was significantly slower (58.7% decrease) than that in control rats; however, the percentages of intravenous dose of unchanged itraconazole excreted in urine for up to 2880 min ($A_{e\ 0–2880\,min}$) were not significantly different between two groups of rats (Table 1).

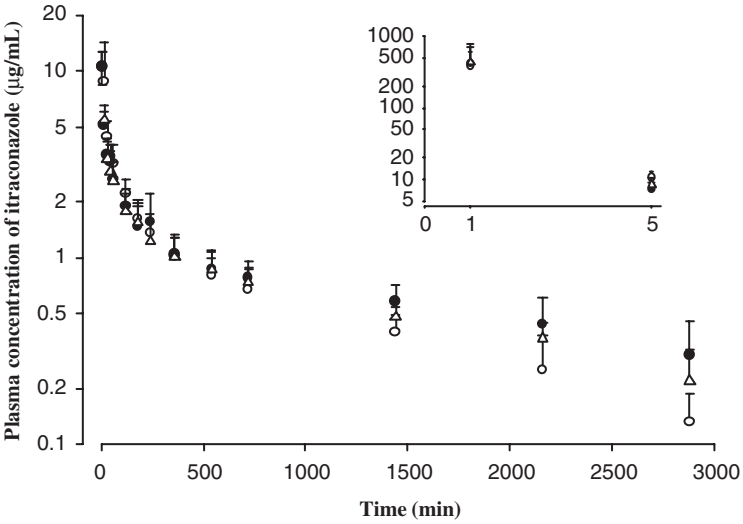

Figure 1. Mean arterial plasma concentration–time profiles of itraconazole after intravenous administration of the drug, 20 mg/kg, to control rats (○, $n = 10$) and rats with PCM (●, $n = 7$) and PCMC (△, $n = 10$). Inset shows the profiles for up to 5 min. Vertical bars represent S.D.

Table 1. Mean (± SD) pharmacokinetic parameters of itraconazole after intravenous administration of the drug, 20 mg/kg, to control rats and rats with PCM and PCMC

Parameters	Control ($n = 10$)	PCM ($n = 7$)	PCMC ($n = 10$)
Body weight (g)	356 ± 42.7[a]	185 ± 17.3	188 ± 12.2
AUC (μg min/ml)	2670 ± 636	3580 ± 713[b]	2980 ± 632
Terminal $t_{1/2}$(min)	865 ± 379	1280 ± 1080	1160 ± 799
MRT (min)	890 ± 409	1940 ± 1270	1460 ± 981
Cl (ml/min/kg)	7.49 ± 1.75[c]	5.59 ± 1.43	6.71 ± 1.54
Cl_r (ml/min/kg)	0.574 ± 0.210[d]	0.379 ± 0.155	0.237 ± 0.310
Cl_{nr} (ml/min/kg)	6.91 ± 1.56[c]	5.11 ± 1.43	6.32 ± 1.57
$A_{e\ 0–2880\,min}$ (% of dose)	8.00 ± 1.28	8.27 ± 3.55	5.52 ± 4.15
$GI_{2880\,min}$ (% of dose)	UD	UD	UD
V_{ss} (ml/kg)	6080 ± 1950	7750 ± 5470	7420 ± 5940

UD: under detection limit.[a] Control group was significantly different ($p < 0.05$) from PCM and PCMC groups.
[b] PCM group was significantly different ($p < 0.05$) from control and PCMC groups.
[c] Control group was significantly different ($p < 0.05$) from PCM group.
[d] Control group was significantly different ($p < 0.05$) from PCMC group.

The V_{ss} values of itraconazole were not significantly different among three groups of rats (Table 1). The itraconazole was under detection limit in gastrointestinal tract measured at 2880 min ($GI_{2880\,min}$) for three groups of rats (Table 1).

The unbound fraction of itraconazole (itraconazole concentration of 5 μg/ml) in rat plasma ($n = 3$) was 0.0034 ± 0.0002 using an ultrafiltration method [19]. The estimated Cl_r of itraconazole based on free (unbound in plasma) fraction in control rats was 169 (0.574 ÷ 0.0034) ml/min/kg; the values were considerably greater than the reported glomerular filtration rate in rats, 5.24 ml/min/kg [20]. The above data indicated that renal secretion of itraconazole was considerable in rats. Considering the Cl_r of itraconazole (Table 1), reported kidney blood flow rate of 36.8 ml/min/kg [20] and hematocrit of approximately 45% [21] in rats, the estimated renal extraction ratio (Cl_r/kidney plasma flow, only for

urinary excretion of unchanged itraconazole) in control rats was 2.84%. The above data indicated that itraconazole was excreted poorly via the kidney in rats (Table 1).

Pharmacokinetics after oral administration of itraconazole

The mean arterial plasma concentration–time profiles of itraconazole after oral administration of the drug, 50 mg/kg, to control rats ($n = 8$) and rats with PCM ($n = 12$) and PCMC ($n = 7$) are shown in Figure 2, and relevant pharmacokinetic parameters are listed in Table 2. Absorption of itraconazole from rat gastrointestinal tract was fast; the plasma concentrations of itraconazole were detected from the first blood sampling time (30 min) for all three groups of rats (Figure 2). After reaching respective peak plasma concentration (338–1070 min), each plasma concentrations of itraconazole seemed to fluctuate for up to 2880 min for each group of rats (Figure 2) suggesting that itraconazole was continuously absorbed from rat gastrointestinal tract and this could be due to high lipophilic nature of itraconazole. The $AUC_{0-2880\,min}$ of itraconazole were not significantly different among three groups of rats (Table 2). After intravenous

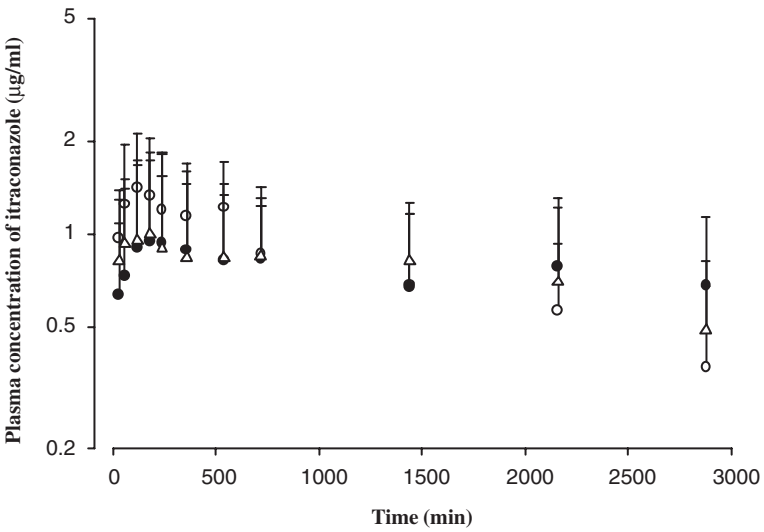

Figure 2. Mean arterial plasma concentration–time profiles of itraconazole after oral administration of the drug, 50 mg/kg, to control rats (○, $n = 8$) and rats with PCM (●, $n = 12$) and PCMC (△, $n = 7$). Vertical bars represent S.D.

Table 2. Mean (± SD) pharmacokinetic parameters of itraconazole after oral administration of the drug, 50 mg/kg, to control rats and rats with PCM and PCMC

Parameters	Control ($n = 8$)	PCM ($n = 12$)	PCMC ($n = 7$)
Body weight (g)	320 ± 73.9[a]	126 ± 31.5	142 ± 33.5
C_{max} (μg/ml)	1.59 ± 0.644	1.00 ± 0.409	1.10 ± 0.703
T_{max} (min)	338 ± 217	595 ± 327	1070 ± 846
$AUC_{0-2880\,min}$ (μg min/ml)	2110 ± 841	2300 ± 1510	2170 ± 1250
Cl_r (ml/min/kg)	0.179 ± 0.169	0.264 ± 0.817	0.0398 ± 0.295
$A_{e\ 0-2880\,min}$ (% of dose)	1.49 ± 1.10[b]	3.47 ± 1.22	0.459 ± 0.410
$GI_{2880\,min}$ (% of dose)	9.07 ± 4.98	6.96 ± 9.18	5.69 ± 2.67
F (%)	31.6	25.7	29.1

[a] Control group was significantly different ($p < 0.05$) from PCM and PCMC groups.
[b] Control group was significantly different ($p < 0.05$) from PCM group.

administration, the AUC of itraconazole in rats with PCM was significantly greater than those in control rats and rats with PCMC (Table 1), however, after oral administration, the $AUC_{0-2880\,min}$ values of itraconazole were not significantly different among three groups of rats (Table 2). This could be due to various factors which could affect the $AUC_{0-2880\,min}$ values of itraconazole after oral administration. After oral administration, the extent of bioavailability and first-pass (gastric, intestinal and/or hepatic) effects should be considered which could be excluded after intravenous administration. The maximum plasma concentration of itraconazole (C_{max}) and time to reach C_{max} (T_{max}) were not significantly different among three groups of rats (Table 2).

Discussion

The *F* values were low; the values were 31.6, 25.7 and 29.1% for control rats and rats with PCM and PCMC, respectively (Table 2). This could be due to considerable first-pass (hepatic, gastric and/or intestinal) effects of itraconazole after oral administration. The hepatic first-pass effect of itraconazole in rats was estimated to be 18.5% [2]. Considerable first-pass effect could be supported by considerably small percentages of itraconazole excreted in urine for up to 2880 min after oral administration; the percentages of intravenous dose of unchanged itraconazole excreted in urine for up to 2880 min were 5.52–8.27% after intravenous administration (Table 1); however, the corresponding values after oral administration were 0.459–3.47% (Table 2).

It was reported that CYP3A4 is involved in the metabolism of itraconazole in human subjects [7, 8] and the expression and mRNA levels of CYP3A23 decreased in rats with PCM [3]. Human CYP3A4 and rat CYP3A1 (CYP3A23) proteins have 73% homology [9]. Hence, it could be expected that in rats with PCM, the plasma concentrations and the resultant AUC of itraconazole could be higher and greater, respectively, than those in control rats. This could be supported by the following results. After intravenous administration of itraconazole to rats with PCM, the plasma levels of itraconazole were considerably higher (Figure 1) and the AUC of itraconazole was significantly greater than those in control rats (Table 1).

It was reported [3] that the altered cytochrome P450 expression by PCM returned to the control level by oral cysteine supplementation (rats with PCMC). Hence, it could be expected that in rats with PCMC, the plasma concentrations and the resultant AUC of itraconazole could be similar to those in control rats. This could be supported by the following results. The AUC of itraconazole after intravenous administration of the drug to rats with PCMC restored fully to the level of control rats. The above result suggests that the modification of dosage regimen of itraconazole may not be required in patients with PCM (such as cancer or AIDS patients), if cysteine is administered to the patients.

Acknowledgements

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