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ORIGINAL ARTICLE

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# Pharmacokinetic and pharmacodynamic study of IST-622, a novel synthetic derivative of chartreusin, by oral administration in a phase II study of patients with breast cancer

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**Abstract** The aim of this study was to analyze the pharmacokinetics and pharmacodynamics (PK/PD) of 6-*O*-(3-ethoxypropionyl)-3′,4′-*O*-exo-benzylidene-chartreusin (IST-622) and its metabolites, and to develop limited sampling models (LSM). Based on the data from 18 patients with breast cancer who were treated orally with 280 or 525 mg/m$^2$ of IST-622 once daily after breakfast for five consecutive days, we analyzed the relationship between the area under the plasma concentration versus time curve (AUC) and toxicities using a sigmoid E-max model and logistic regression. Plasma concentrations of IST-622 and its metabolites, 3′,4′-*O*-exo-benzylidene-chartreusin (A-132) and 3″-demethyl-3′,4′-*O*-exo-benzylidene-chartreusin (A-132M), were measured at 1, 2, 4, 8 and 24 h after administration on day 1. The AUC was calculated using the trapezoidal method. We also developed a LSM using stepwise linear regression analysis. IST-622 was detected in very few patients, and its concentration was very low and could be disregarded. It was suggested that meals promoted absorption of IST-622. AUCs of A-132 plus A-132M showed a better correlation with the rates of decrease and nadir counts of leukocytes, neutrophils and platelets than the AUC of each metabolite separately. Patients with the sum of AUCs more than 70 μg·h/ml showed severe myelotoxicities. Moreover, logistic regression analysis showed that grade 4 myelotoxicities would be seen in 30% of patients at an AUC of 65 μg·h/ml. We also developed an unbiased and precise LSM: $AUC_{0-24h} = C_{8h} \times 17.6 - 0.95$, where $C_{8h}$ denotes the sum of plasma concentrations of A-132 and A-132M. Myelotoxicities showed a good correlation with $AUC_{0-24h}$, and based on the results, it was decided that the target AUC was 65 μg·h/ml. The LSM was very convenient for estimating $AUC_{0-24h}$ and sufficiently accurate. These results show the possibility of predicting toxicities and dose adaptation for interpatient variability using LSM.

**Keywords** Chartreusin · IST-622 · Limited sampling model · Pharmacokinetics · Pharmacodynamics

## Introduction

Chartreusin, an antibiotic produced by *Streptomyces chartreusis*, has been reported to show significant antitumor activity against several mouse tumors in vivo when the intraperitoneal route was used for both tumor cell inoculation and drug administration [6]. However, chartreusin was not chosen for clinical trials because of its unfavorable pharmacokinetics including very rapid biliary excretion after intravenous administration and very slow gastrointestinal absorption [6]. A series of 3′,4′-*O*-substituted derivatives of chartreusin were synthesized and their antitumor effects examined. Among them, 3′,4′-*O*-exo-benzylidene-chartreusin (A-132) was found to be effective against B16 melanoma following both intraperitoneal and intravenous administration. By further chemical modification, more promising derivatives such as 6-*O*-(3-ethoxypropionyl)-3′,4′-*O*-exo-benzylidene-chartreusin (IST-622) were obtained. Oral administration of IST-622 results in a more prominent effect than A-132 [3, 10].

IST-622 is rapidly metabolized to A-132 and 3″-demethyl-3′,4′-*O*-exo-benzylidene-chartreusin (A-132M) (Fig. 1), and these metabolites as well as IST-622 show antitumor activity against various tumors in vitro and in vivo. A-132 and A-132M show similar antitumor activities. It is thought that their antitumor activity depends on inhibition of not only topoisomerase II but also topoisomerase I in high concentrations. It is suggested that IST-622 and its metabolites stabilize complexes of DNA and topoisomerases [4].

In a previous phase I study, IST-622 was orally administered before breakfast for five consecutive days. Dose-limiting toxicities were hematologic, and included neutropenia and thrombocytopenia. Nonhematologic toxicities were mild. The estimated recommended dose for phase II study was from 525 to 700 mg/m$^2$. Patients was given IST-622 after breakfast in step I of this phase II study. Although six patients received 525 mg/m$^2$ of IST-622 after breakfast, the frequency of toxicities and the area under the plasma concentration versus time curves (AUC) were higher than in the phase I study. The difference was thought to be that meals promote the absorption of IST-622. Therefore, in step II of this phase II study, patients received 280 mg/m$^2$ of IST-622 after breakfast.

The objectives of this study were to analyze the pharmacokinetics and pharmacodynamics (PK/PD) and to develop a limited sampling model for estimating toxicities and for adopting individual doses using the data from the phase II study.

## Materials and methods

We used the data from step I and II of the phase II study of IST-622 for breast cancer. The study was approved by the ethics review boards of the institutions, and performed from August 1996 to June 1998. Written informed consent was obtained in advance. Patients were treated orally with IST-622 once a day after breakfast for five consecutive days. Drug administration was repeated every 4 weeks. A group of 21 patients with adequate organ function and advanced or recurrent disease participated in this study. Six patients were given 525 mg/m$^2$ of IST-622 (step I) and 15 patients were given 280 mg/m$^2$ of IST-622 (step II). Complete PK/PD data were obtained from 18 patients in phase II. We could not obtain complete pharmacokinetic data from three patients because of problems including difficulty in venous access. The characteristics of the 18 patients are shown in Table 1.

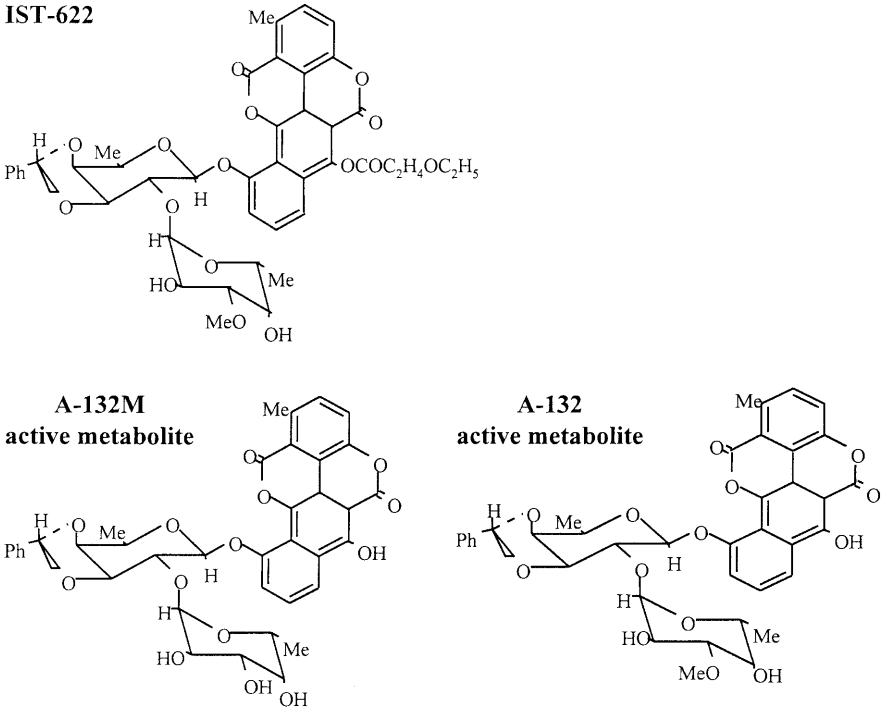


**Fig. 1.** Chemical structures of IST-622 and its metabolites, A-132 and A132M

**Table 1.** Characteristics of the 18 patients available for PK/PD analysis

| | |
|---|---|
| Age (years) | |
| Median | 58.5 |
| Range | 35–75 |
| Dose ($mg/m^2$) | |
| 280 | 13 |
| 525 | 5 |
| Body surface area ($m^2$) | |
| Median | 1.48 |
| Range | 1.28–1.79 |
| Performance status | |
| 0 | 14 |
| 1 | 3 |
| Record missing | 1 |
| Number of courses | |
| Median | 3 |
| Range | 1–5 |
| Prior chemotherapy (Y/N) | 18/0 |

### Measurement of plasma concentration for IST-622, A-132 and A-132M

Blood samples for the pharmacokinetic study were taken 1, 2, 4, 8 and 24 h after oral administration on the first day of the first course. Plasma concentrations of IST-622, A-132 and A-132M were measured at the Institute of Biological Science, Mitsui Pharmaceuticals (Tokyo, Japan) using high-performance liquid chromatography. Plasma was injected onto an ODS L-column, and metabolites were eluted isocratically using 20 m*M* acetate buffer (pH 4.0)/$CH_3CN$ (4:6 v/v) at a flow rate of 1.2 ml/min. Detection was carried out by fluorescence with excitation at 265 nm and emission at 450 nm.

### PK/PD analysis

We calculated the AUCs of A-132, A-132M and the sum of both using the trapezoidal method. IST-622 was detected in very few patients, and its concentration was very low and could be disregarded. Toxicities were evaluated according to JCOG criteria [11]. In order to analyze the relationship between AUCs and myelotoxicities of the first courses, we used (inhibitory) sigmoid E-max models. These calculations were done using the WinNonlin ver 1.1 program (Scientific Consulting, Apex, N.C.). We also analyzed the relationship between AUCs and myelotoxicities using logistic regression analysis using the Windows SAS ver 6.12 program (SAS Institute, Cary, N.C.).

### Developing a limited sampling model

We randomized the data into two groups (training data set and validation data set). A limited sampling model was developed using the training data set, and was confirmed as unbiased and precise using the validation data set. The modeling was done with the stepwise multiple linear regression method using the SPSS 6.1.3J program (SPSS, Chicago, Ill.). After the model had been confirmed as unbiased and precise using mean prediction error (MPE) and root mean squared error (RMSE) [8], remodeling was performed using all the data in order to make a more precise model.

## Results

### PK/PD analysis

We analyzed the PK/PD data using sigmoid E-max models. The AUCs of A-132, A132M and the sum of both were adopted as pharmacokinetic parameters, and percentage decrements and nadir counts in leukocytes, neutrophils and platelets were adopted as pharmacodynamic parameters. Correlations between the observed and predicted values in the sigmoid E-max models are shown in Table 2. The AUCs of A-132, A-132M and the sum of both were significantly correlated with nadir counts and decrements in leukocytes, neutrophils and platelets. The total AUC of A-132 and A-132M showed the most stable correlations and *P*-values among the AUCs. The nadir count showed a better correlation than the percentage decrement, especially absolute neutrophil count (ANC). On the other hand, the correlations were similar between nadirs and decrements for leukocytes and platelets. Moreover, the sigmoid E-max models of decrements and nadir counts showed that patients with AUCs of 70 μg·h/ml had severe (grade 4) leukopenia, neutropenia and thrombocytopenia (Figs. 2, 3 and 4).

Using logistic regression analysis we analyzed the relationships between total AUCs and the probability of one or more grade 4 myelotoxicities (leukopenia, neutropenia, thrombocytopenia). There was a tendency for a correlation between myelotoxicities and AUCs. According to this analysis, 30% of patients with an AUC of 65 μg·h/ml will have grade 4 myelotoxicities (Fig. 5).

### Limited sampling model

From the training data set, we developed the following models: $AUC_{0-24h}(\mu g \cdot h/ml) = C_{8h} \times 17.3 + 0.21$ or $AUC_{0-24h} = C_{8h} \times 10.1 + C_{24h} \times 9.5 + 9.6$, where $C_{8h}$ and $C_{24h}$ denote total plasma concentrations (in μg/ml) of A-132 and A-132M 8 h and 24 h after oral intake. These models were confirmed as unbiased and precise by

**Table 2.** Correlations between observed and predicted values from PK/PD analyses using the sigmoid E-max model. An inhibitory sigmoid E-max model was used for analyses between AUCs and WBC, ANC and platelet nadirs

| AUC | WBC | | ANC | | Platelets | |
|---|---|---|---|---|---|---|
| | | *P* value | | *P* value | | *P* value |
| A132 + A132M | | | | | | |
| Nadir | 0.807 | < 0.001 | 0.866 | < 0.001 | 0.768 | < 0.001 |
| Percent decrement | 0.795 | < 0.001 | 0.535 | 0.039 | 0.855 | < 0.001 |
| A132 | | | | | | |
| Nadir | 0.803 | < 0.001 | 0.850 | < 0.001 | 0.678 | 0.003 |
| Percent decrement | 0.753 | 0.001 | 0.505 | 0.056 | 0.848 | < 0.001 |
| A132M | | | | | | |
| Nadir | 0.757 | 0.001 | 0.815 | < 0.001 | 0.697 | 0.002 |
| Percent decrement | 0.794 | < 0.001 | 0.540 | 0.039 | 0.815 | < 0.001 |

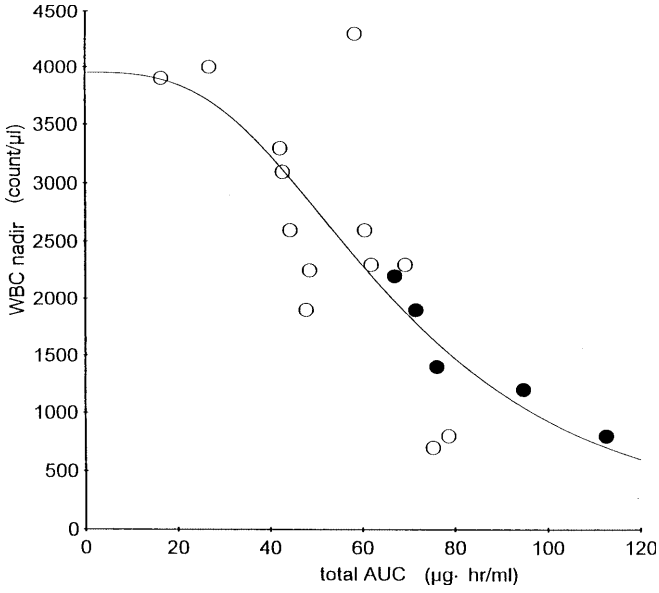


**Fig. 2.** Relationship between nadir WBC counts and total AUCs with inhibitory sigmoid E-max regression (*open circles* 280 mg/m$^2$, *filled circles* 525 mg/m$^2$)

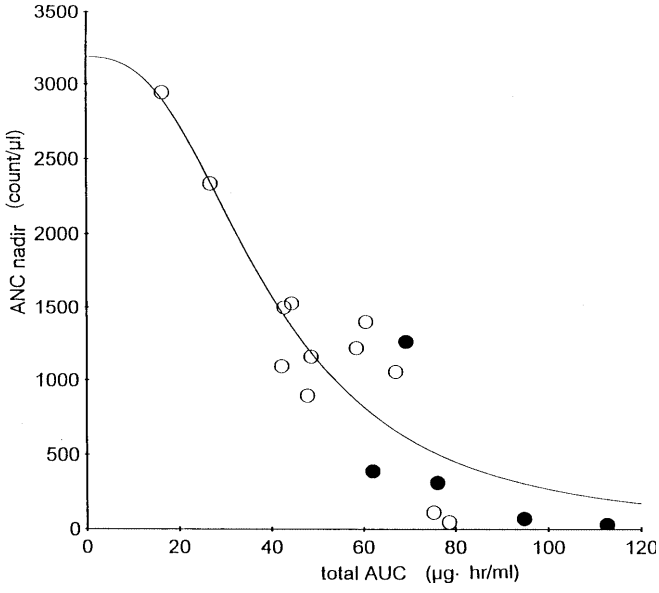


**Fig. 3.** Relationship between nadir ANC counts and total AUCs with inhibitory sigmoid E-max regression (*open circles* 280 mg/m$^2$, *filled circles* 525 mg/m$^2$)

the validation data set. The mean values of %MPE and %RMSE were 2.71 and 11.01 for a one-sample model, and –0.48 and 16.03 for a two-sample model. These results show that the one-sample model was sufficiently accurate. We developed a new one-sample model using all the pharmacokinetic data in order to produce a more accurate model: $AUC_{0-24h} = C_{8h} \times 17.6 - 0.95$ ($r = 0.937$). The relationship between the observed AUCs and the estimated AUCs by the one-sample model is shown in Fig. 6. The values of %MPE and %RSME were 1.92 and 10.3, respectively.

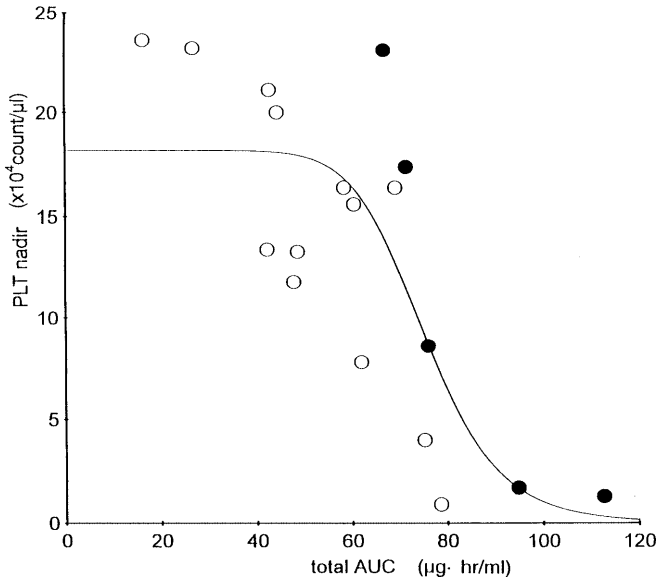


**Fig. 4.** Relationship between nadir platelet counts and total AUCs with inhibitory sigmoid E-max regression (*open circles* 280 mg/m$^2$, *filled circles* 525 mg/m$^2$)

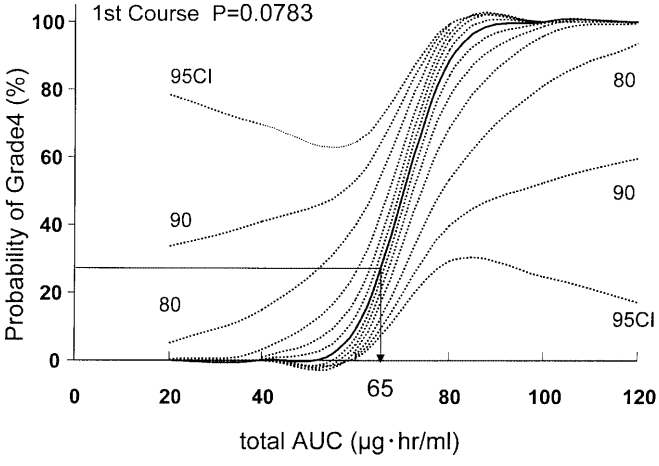


**Fig. 5.** Estimation of AUC with 30% probability of grade 4 myelosuppression using logistic regression analysis

## Discussion

It is most important that we control the adverse effects of anticancer drugs. Myelotoxicities are the commonest adverse effect in anticancer chemotherapy, and the effects of IST-622 are no exception. Many investigators have reported clinical PK/PD analyses of anticancer agents, and the correlations between pharmacokinetic parameters and pharmacodynamic reactions in various kinds of agent are understood. We know that AUC correlates with antitumor effects and adverse effects for many agents. For example, many PK/PD analyses of carboplatin have found correlations between AUC and pharmacodynamic reactions. Higher AUCs (over 7 mg/ml·min) cause severe myelotoxicities and produce the

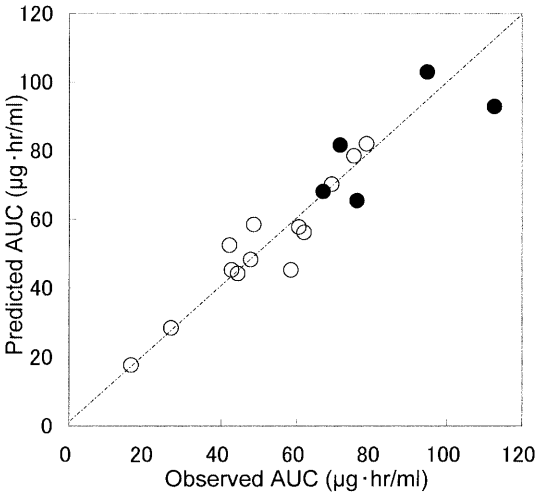


**Fig. 6.** Relationship between observed AUCs and estimated AUCs by the one-sample model. $AUC_{0-24h} = C_{8h} \times 17.6 - 0.95$ ($r = 0.937$). The *broken line* is the line of identity (*open circles* 280 mg/m$^2$, *filled circles* 525 mg/m$^2$)

maximum response rate. On the other hand, lower AUCs (less than 5 mg/ml·min) decrease the response rate in ovarian cancer [2]. We can also adjust the dose of carboplatin using glomerular filtration rate determined using the Calvert formula for a targeted AUC [1]. Therapeutic drug monitoring is also effective for dose adjustment of oral etoposide because of its narrow therapeutic window of plasma concentration [5, 7, 9].

Although many PK/PD analyses of various anticancer drugs have been performed, such analyses do not always contribute to the adjustment of doses in individuals. There are two major reasons why we cannot use these analyses in clinical practice. One is the wide range of pharmacodynamic reactions among individuals, and the other is the difficulty in dose adjustment using pharmacokinetic parameters because for many drugs such parameters cannot easily be predicted.

In this study we found good PK/PD correlations for the metabolites of IST-622. We seek to target the dose using nadir counts rather than percentage decrement because in clinical practice evaluation of hematologic toxicities is done using nadir counts. Indeed, nadir counts as well as percentage decrement showed good correlations with AUCs. There was a narrow range of pharmacodynamic reactions among individuals for IST-622, and we were able to estimate that the target AUC is 65 μg·h/ml. This result means that we can control the adverse effects of IST-622 if the AUCs of its metabolites are controlled.

We also found that a limited sampling model of the metabolites of IST-622 was very useful for estimating AUCs. For most drugs it is not possible to estimate individual pharmacokinetic parameters such as AUC or clearance before administration. However, for IST-622 it is possible to predict the pharmacokinetic parameters and adjust the dose for a target AUC by testing its administration in advance of therapy. The results of this clinical trial suggest that low doses administered for only 1 day will cause very few adverse effects. This administration testing will involve much lower AUCs than therapeutic administration for five consecutive days. Moreover, we do not need therapeutic doses of IST-622 for administration testing. On the premise that clearance does not change in the same patient, a target dose is determined using two equations: $clearance = targetdose/targetAUC$ and $clearance = testdose/testAUC$. The AUCs of A132 plus A132M were shown to increase dose-dependently in a previous phase I study (data not shown).

From these results it seems clear that the risk of therapeutic administration without prior testing is higher than the risk of low-dose testing administration. AUC-targeted administration is highly recommended for the safety of IST-622 therapy, and the limited sampling method will be a useful tool for estimating AUCs and determining dose adjustments.

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