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Pharmacokinetic properties of indinavir in rat: some limitations of noncompartmental analysis

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Abstract

Background: Compartmental as well as noncompartmental analyses are used routinely in pharmacokinetic analysis. *Materials and methods*: Pharmacokinetic parameters of the anti-HIV agent, indinavir, have been determined in six male rats applying both the compartmental and the noncompartmental analysis. *Results and discussion*: A very slow declining phase was found in the indinavir plasma concentration profile using an extended sampling time period and applying a sensitive high-performance liquid chromatography assay method. This apparent terminal elimination phase can cause some significant errors when applying noncompartmental kinetic analysis to the data, with mean residence time (MRT) (544.2 ± 123.2 minutes), total systemic clearance (12.0 ± 2.1 mL/min/kg), and steady-state volume of distribution (V_d (ss)) (6.4 ± 1.0 L/kg) being highly different from the results of compartmental kinetic analysis (MRT, Cl_{total}, and V_d (ss) values of 23.7 ± 5.9 minutes, 35.18 ± 5.1 mL/min/kg, and 0.84 ± 0.28 L/kg, respectively). The parameters estimated by our noncompartmental approach were also significantly different from the results of the same type of data analysis reported in the literature. *Conclusion*: The differences in parameter estimations, while being a result of the extended plasma sampling period, which is recommended in noncompartmental analysis, support the priority of applying the compartmental analysis approach in the similar cases with some pre-assumptions, mainly ignoring the final apparent terminal elimination phase(s), very deep tissue, which involves very low drug concentrations.

Key words: *Compartmental analysis; HIV protease inhibitors; indinavir; noncompartmental analysis; pharmacokinetics*

Introduction

The development of HIV protease inhibitors has revolutionized antiretroviral therapy in recent years[1–3]. The use of 'highly active antiretroviral therapy' (HAART), which includes at least one HIV protease inhibitor in combination with nucleoside reverse transcriptase inhibitors, has resulted in a substantial improvement in acquired immune deficiency syndrome (AIDS) surrogate markers in the immune system[4,5]. Indinavir is a potent HIV protease inhibitor with a widespread use in the treatment of AIDS[6,7].

A high degree of correlation between plasma/tissue concentrations of indinavir and its antiretroviral effect has been demonstrated[8–11]. Therefore, the importance of pharmacokinetic studies on this drug is evident. A series of investigations have been carried out on the pharmacokinetics of indinavir in animal models[12–18], all of which involve the application of the widely used noncompartmental analysis. Noncompartmental (model-independent) pharmacokinetic analysis is primarily based on the calculation of the area under the plasma concentration–time curve (AUC) of a drug following its systemic administration to humans/animals, regardless of the phases of drug disposition[19,20]. Consequently, this type of analysis results in the estimation of 'overall' pharmacokinetic parameters (e.g., Cl_{total} and V_d (ss)). Contrarily, in the compartmental approach, the time course of drug concentration in plasma is divided into different phases and the kinetic parameters are determined for each phase from which the pharmacokinetic parameters of the drug can be calculated[21,22].

In this study, both compartmental and noncompartmental analysis are applied on a set of plasma concentration data obtained upon intravenous administration of indinavir to rats. Based on the results, some serious

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limitations of the application of noncompartmental pharmacokinetic approach are discussed.

Materials and methods

Materials

Indinavir sulfate (ethanol solvate form, MW 757.9) was kindly donated by Merck Research Laboratories (Rahway, NJ, USA). All other reagents used were of analytical or high-performance liquid chromatography (HPLC) purity grades, as needed.

Animals

Six male Sprague–Dawley rats (Charles River, St. Constant, Quebec, Canada) weighing between 280 and 300 g were used in this study. The animals were kept in standard cages with a free access to water and standard rat chow ad libitum. A 12-hour day–night cycle was used with lights on at 8:00 am. The protocol for the animal experiments was reviewed and approved by the University of Toronto Animal Care Committee. The animals were cared for in accordance with the guidelines of the Canadian Council on Animal Care.

Drug administration

The day before the experiments, the rats were anesthetized by intraperitoneal injection of a ketamine-xylazine cocktail (ketamine 100 mg/kg and xylazine 10 mg/kg), and a polyethylene-silicone rubber cannula was implanted in the right jugular vein according to a standard method[23]. The rats were then left overnight for complete recovery while kept singly in smaller cages.

In the day of the experiment, a 5-mg/kg dose of indinavir sulfate dissolved in a saline-propylene glycol-ethanol vehicle (5:4:1, v/v/v; 5 mg/mL) was injected to each rat through the cannula. The animals remained unrestrained during the entire sampling time. At 0 and 1, 5, 10, 30, 60, 120, 180, 240, 360, and 420 minutes after drug injection, blood samples (0.4 mL) were collected through the cannula to pre-heparinized 1.5-mL polypropylene microtubes and were replaced immediately by injection of the same volume of sterile saline. The blood samples were then centrifuged at $1000 \times g$ for 10 minutes and the plasma fractions were separated and kept frozen at −70°C until analyzed.

Drug assay

Indinavir plasma concentrations were determined using a simple and validated HPLC method developed in our laboratory[24]. Briefly, to 0.15 mL of plasma, 0.5 mL of NaOH 1M solution and 4 mL of diethyl ether were added and the resulting mixture was shaken 15 minutes and, then, centrifuged at $3000 \times g$ for 10 minutes. The organic layer was separated in a hexane-dry ice bath and back-extracted by adding 0.1 mL of phosphoric acid (25 mM). After centrifuging at $3000 \times g$ for 10 minutes, the major part of the organic layer was separated by aspiration and the test tubes were left at room temperature for 30 minutes to evaporate the remainder of ether. Finally, 50 µL of the aqueous layer was injected to the chromatograph.

A mixture of acetic acid aqueous solution (50 mM) and acetonitrile (52:48, v/v) with a pH of 4.8, adjusted by adding KOH 1M solution, was delivered using an HPLC pump (model 1050; Hewlett-Packard, Santa Clara, CA, USA) with a flow rate of 1.2 mL/min. The analyte was separated by a Zorbax SB-C18 column (75×4.6 mm, particle size 3.5 µm; Agilent Technologies, Santa Clara, CA, USA) and detected using a UV-detector (model 1050; Hewlett-Packard) at wavelength of 260 nm. The sample injection was made by an auto-injector (model 715 ULTRA WISP; Waters, Milford, MA, USA). The method resulted in linear responses throughout the concentration range of 0.05–32 µM of indinavir (r^2 = 0.998) with the average intra- and inter-run variations of 3.40% and 6.87% (N = 3) over the studied concentration range, respectively.

Pharmacokinetic analysis

Compartmental analysis

The plasma concentration–time data were fit to the biexponential decline curve using SigmaPlot 5.0 software (SPSS Inc., Chicago, IL, USA) and the corresponding slopes and intercepts of the elimination and distribution phases were determined. The rate constant of the terminal phase (λ_z) was determined by linear regression analysis of the terminal portion of the plasma concentration–time curves.

The following set of equations[19–21] were used in the calculation of compartmental pharmacokinetic parameters of the drug, considering A and B as intercepts and $\alpha/2.303$ and $\beta/2.303$ as slopes of the distribution and elimination lines, respectively:

$$C_0 = A + B$$

$$V_c = \text{dose}/C_0$$

$$V_d(\text{ext}) = \text{dose}/B$$

$$V_d(\beta) = \text{Cl}_{\text{total}}/\beta$$

$$V_d(\text{ss}) = V_c(K_{12} + K_{21})/K_{21}$$

$$K_{\text{ave}} = \text{Cl}_{\text{total}}/V_d(\text{ss})$$

Compartmental mean residence time (MRT_{comp}) = $1/K_{ave}$
Mean residence time in central compartment (MRT_c) = $(AUC_{0\to\infty})_{comp}/C_0$
Mean residence time in tissue compartment (MRT_t) = $MRT_{comp} - MRT_c$
Mean residence time in deep tissue compartment (MRT_{dt}) = $MRT_{noncomp} - MRT_{comp}$

$$Cl_{total} = V_c \cdot K_{10}$$

$$K_{21} = A\beta + B\alpha/A + B$$

$$K_{10} \cdot K_{21} = \alpha \cdot \beta$$

$$K_{21} + K_{12} + K_{10} = \alpha + \beta$$

$$(AUC_{0\to\infty})_{comp} = A/\alpha + B/\beta$$

Noncompartmental analysis

The area under the plasma concentration–time curve was calculated for the interval between 0 and 420 minutes ($AUC_{0\to420}$) using the trapezoidal rule and between 0 and ∞ ($AUC_{0\to\infty}$) using the formula: $AUC_{0\to\infty} = AUC_{0\to420} + C_{420}/\lambda_z$ [20], where C_{420} is the plasma concentration of the drug at 420 minutes and λ_z is the decline rate constant for the terminal log-linear portion of the multi-exponential curve.

The area under the first moment of plasma concentration–time curve was calculated for the interval between 0 and 420 minutes ($AUMC_{0\to420}$) using the $C_t \cdot t$ versus t data and applying the trapezoidal rule and between 0 and ∞ ($AUMC_{0\to\infty}$) by the formula[20]

$$AUMC_{0\to\infty} = AUMC_{0\to420} + C_{420}\,(420)/\lambda_z + C_{420}/\lambda_z^2$$

Using the above-defined parameters, the following equations[19,20] were used for the determination of the other noncompartmental pharmacokinetic parameters:

Noncompartmental mean residence time ($MRT_{noncomp}$) = $AUMC_{0\to\infty}/AUC_{0\to\infty}$

$$Cl_{total} = dose/AUC_{0\to\infty}$$

$$V_d(ss) = MRT_{noncomp} \cdot Cl_{total}$$

$$K_{ave} = 1/MRT_{noncomp}$$

Using the same set of calculations, another series of noncompartmental parameters were calculated considering the data from 0 to 240 minutes.

Statistical analysis

The significance of differences between the pharmacokinetic parameters was determined using the statistical ANOVA or unpaired *t*-test whenever applicable. A *P*-value < 0.05 was considered significant. For evaluation of the pairwise significant differences, a Tukey's post hoc test was performed on the data.

Results and discussion

The mean plasma concentration–time curve of indinavir following the administration of a 5-mg/kg intravenous dose of the drug to six rats is shown in Figure 1. The pharmacokinetic profile can be described as a three-compartment-open model consisting of a central, a tissue, and a deep tissue compartment. In fact, by extending the sampling time up to 420 minutes and using a sensitive assay method, we found that there is a distinct terminal very slow declining phase in the plasma concentration–time curve of indinavir in rats. In previous studies[13,14,16–18], because of the limited sampling time, this terminal phase has been 'lumped' with the second exponential phase in the determination of the elimination rate constant. This, in turn, has led to an estimation of some longer elimination half-life than the one we observed in this study (28.5 ± 2.2 versus 21.1 ± 4.5 minutes). As this terminal phase includes only about 2% of the drug dose (judged by comparing the plasma concentrations at 1- and 120-minute time points), we did not consider this phase in our compartmental analysis to avoid the considerable degree of error in the calculation of the pharmacokinetic parameters. Consequently, the second exponential phase of the

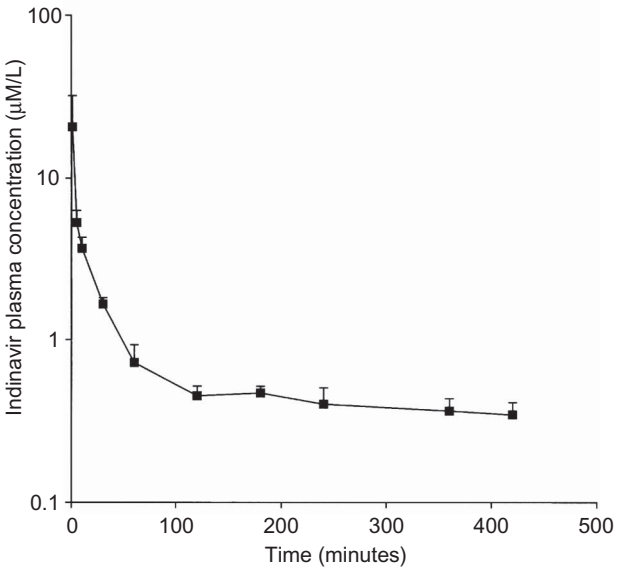

Figure 1. Plasma concentration–time curve of indinavir in six male Sprague–Dawley rats upon IV administration of 5 mg/kg indinavir sulfate.

concentration profile was regarded as the 'effective elimination phase' in this study with the following equation defining the plasma concentration–time relationship, $C_t = Ae^{-\alpha t} + Be^{-\beta t}$.

With this initial assumption, the compartmental pharmacokinetic analysis was carried out on the plasma concentration data. Results, as listed in Table 1, are indicative of a very rapid and extensive distribution phase of the drug in rats as reflected by the high α (38.8 ± 12.5 h^{-1}) and V_d (ss) (842.4 ± 278.7 mL/kg) values, respectively (Table 1). In addition, the elimination rate constant of the drug is also high in this species (β = 2.0 ± 0.4 h^{-1}). These findings are in good agreement with the previously reported high systemic clearance (average 90 mL/min/kg) of the drug in rat[13,14,16–18]. The rapid distribution and elimination processes of the drug are also reflected by the high values of k_{12} (22.2 ± 10.1 h^{-1}) and k_{10} (10.8 ± 5.3 h^{-1}), respectively. On the other hand, the high value of k_{21} (7.8 ± 2.7 h^{-1}) represents a rapid back-distribution of drug, thereby indicating a fast equilibrating tissue distribution (a relatively short distribution phase). We also calculated the MRTs of the drug in the central, tissue, and deep tissue compartments (Table 1). These values (i.e., 6.5 ± 2.6, 17.2 ± 6.6, and 523.6 ± 120.6 minutes for central, tissue, and deep tissue compartments, respectively), again, are representative of the fast distribution and elimination of the drug with a very slow apparent terminal elimination rate. The apparent terminal phase, which was not considered in the compartmental analysis, shows a very slow decline rate, suggesting a small amount of the drug can redistribute from a very deep tissue reservoir into the blood circulation (λ_z and MRT_{dt} values of 0.09 ± 0.02 h^{-1} and 523.6 ± 120.6 minutes, respectively; Table 1).

Table 1. Pharmacokinetic parameters of indinavir in male Sprague–Dawley rats applying compartmental analysis (420-minute sampling period) (6 rats).

Parameter	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Mean (SD)
A (μM)	22.12	77.34	19.67	19.39	9.09	43.70	31.89 (25.00)
B (μM)	3.57	4.55	4.36	5.74	6.34	4.80	4.89 (1.00)
C_0 (μM)	25.69	81.89	24.06	25.13	15.43	48.5	36.78 (24.69)
α (h^{-1})	52.96	46.36	26.63	42.76	20.54	43.78	38.84 (12.49)
$T_{1/2}$, α (minutes)	0.785	0.954	1.562	0.972	2.023	0.9497	1.21 (0.48)
β (h^{-1})	1.482	1.920	1.908	2.274	2.820	1.866	2.04 (0.45)
$T_{1/2}$, β (minutes)	28.06	21.66	21.79	18.28	14.74	22.28	21.14 (4.45)
λ_z (h^{-1})	0.089	0.073	0.105	0.067	0.125	0.083	0.09 (0.02)
$T_{1/2}$, λ_z (hours)	7.79	9.49	6.57	10.40	5.51	8.30	8.01 (1.81)
V_c (mL/kg)	256.91	80.59	274.31	262.63	427.74	136.08	239.71 (121.19)
V_d (ext) (mL/kg)	1843.74	1450.55	1513.76	1149.83	1041.01	1375.00	1395.65 (284.38)
V_d (β)(mL/kg)	491.26	850.94	1143.08	974.67	869.15	991.00	886.68 (220.14)
V_d (ss)(mL/kg)	1348.78	514.20	883.89	834.17	746.64	726.82	842.42 (278.73)
Cl_{total} (mL/min/kg)	38.88	27.23	36.35	36.94	40.85	30.82	35.18 (5.15)
K_{ave} (h^{-1})	1.73	3.18	2.47	2.66	3.28	2.54	2.64 (0.56)
K_{12} (h^{-1})	36.72	23.62	14.20	25.07	7.53	26.05	22.20 (10.15)
K_{21} (h^{-1})	8.64	4.39	6.39	11.52	10.10	6.01	7.84 (2.71)
K_{10} (h^{-1})	9.08	20.27	7.95	8.44	5.73	13.59	10.84 (5.29)
$AUC_{0\to\infty}$ (μM/min)	169.59	242.20	181.43	178.66	161.44	214.23	191.26 (30.78)
MRT_{comp} (minutes)	34.68	18.87	24.29	22.56	18.29	23.62	23.72 (5.91)
MRT_c (minutes)	6.60	2.96	7.54	7.11	10.46	4.42	6.52 (2.61)
MRT_t (minutes)	28.08	15.91	16.75	15.45	7.83	19.2	17.20 (6.59)
MRT_{dt} (minutes)	540.13	610.24	389.82	690.17	384.521	526.55	523.57 (120.64)

C_0, the theoretical plasma concentration in time zero; A, intercept of the distribution regression line; B, intercept of the elimination regression line; α, distribution rate constant; β, elimination rate constant; λ_z, terminal elimination rate constant; V_c, volume of distribution in the central compartment; V_d (ext), extrapolated volume of distribution; V_d (β), beta volume of distribution; V_d (ss), steady-state volume of distribution; MRT_{comp}, compartmental mean residence time; MRT_c, mean residence time in central compartment; MRT_t, mean residence time in tissue compartment; MRT_{dt}, mean residence time in deep tissue compartment; Cl_{total}, total systemic clearance; K_{12}, rate constant of drug transfer from central to peripheral (tissue) compartment; K_{21}, rate constant of drug transfer from peripheral to central compartment; K_{10}, rate constant of drug elimination from central compartment; $(AUC_{0\to\infty})_{comp}$, area under plasma concentration–time curve from 0 to ∞ determined using the compartmental approach; $AUC_{0\to420}$, area under the plasma concentration–time curve from 0 to 420 minutes; $AUC_{0\to240}$, area under the plasma concentration–time curve from 0 to 240 minutes; $AUC_{0\to\infty}$, area under the plasma concentration–time curve from 0 to ∞; $AUMC_{0\to420}$, area under the first moment of plasma concentration–time curve from 0 to 420 minutes; $AUMC_{0\to240}$, area under the first moment of plasma concentration–time curve from 0 to 240 minutes; $AUMC_{0\to\infty}$, area under the first moment of plasma concentration–time curve from 0 to ∞; $MRT_{noncomp}$, noncompartmental mean residence time; K_{ave}, average elimination rate constant.

As it was indicated earlier, all the previous pharmacokinetic studies of indinavir in rat involved noncompartmental analysis. Therefore, we undertook a noncompartmental analysis of the data to compare the results with both compartmental analysis data and the ones reported in the literature. Results from the noncompartmental analysis of the plasma concentration data are shown in Table 2 (420-minute sampling time) and Table 3 (240-minute sampling time).

Comparison of our noncompartmental analysis of indinavir data to the results of the same type of analysis in the literature reveals a significant difference in all the parameters (Table 4). We found significantly higher MRT and V_d (ss) and lower Cl_{total} values in our study ($P < 0.01$). Based on the Tukey's post hoc test, all the data set pairs were different from each other. This can possibly be explained by different duration of sampling period as well as different assay methods, considering that all the data in the literature have been published by the same research group[13–17]. To clarify the relative contribution of these two factors, a series of noncompartmental analyses were carried out on the data omitting the final two time points (i.e., considering the total sampling period of 240 minutes) (Table 3). Although closer, the results still remain significantly different from the ones reported in the literature ($P < 0.01$; Table 4). Another factor that could explain the difference in the results is the analytical specification of the drug assay method. It is clear that only a small degree of difference in assay sensitivity may result in a considerable difference in drug concentrations measured at the late sampling times in which the drug concentration is usually low. These different concentrations, in turn, could ultimately have a significant effect on AUC estimation. Comparing the pharmacokinetic profiles obtained in this study with the ones reported in the literature[13,14], we found that the plasma concentrations at similar time points are considerably different, especially at the late time points. We believe that these differences can mainly be the result of different assay methods. Considering the fact that the basis for noncompartmental analysis is

Table 4. Comparative noncompartmental pharmacokinetic parameters of indinavir determined in this study with parameters reported in literature.

Study	Sampling time (minutes)	V_d (ss) (L/kg)[a]	Cl_{total} (mL/min/kg)[a]
This study	240	4.38 (0.45)[b]	16.99 (3.62)
This study	420	6.40 (0.99)	12.03 (2.09)
Lin et al.[13]	240	2.24 (0.46)	107.0 (31.0)
Lin et al.[14]	180	2.17 (0.84)	89.0 (18.0)
Chiba et al.[15]	NR[c]	2.2 (0.46)	100 (21.1)
Lin et al.[17]	300	2.17 (0.84)	79.0 (18.0)
Vacca et al.[18]	NR	2.17 (0.84)	79.0 (18.0)

[a]Based on the Tukey's post hoc test, the values for two sampling times in this study are significantly different from each other as well as the values reported in individual references included in the Table ($P < 0.01$). [b]Mean (SD). [c]NR, not reported.

Table 2. Pharmacokinetic parameters of indinavir in male Sprague–Dawley rats applying noncompartmental analysis (420-minute sampling period).

Parameter	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Mean (SD)
$AUC_{0\to420}$ (μM/min)	301.10	366.91	276.97	302.64	351.61	322.07	320.22 (33.80)
$AUC_{0\to\infty}$ (μM/min)	557.28	679.29	414.11	598.16	556.37	560.62	560.97 (86.01)
$AUMC_{0\to420}$ (μM/h^2)	11.12	10.96	8.25	9.72	11.28	9.78	10.19 (1.17)
$AUMC_{0\to\infty}$ (μM/h^2)	88.98	118.71	45.27	117.71	62.25	85.68	86.43 (29.35)
V_d (ss)(L/kg)	6.81	6.11	6.38	7.81	4.78	6.48	6.40 (0.99)
MRT (minutes)	574.81	629.11	400.06	708.46	402.81	550.17	544.24 (123.23)
Cl_{total} (mL/min/kg)	11.84	9.72	15.94	11.03	11.86	11.77	12.03 (2.09)
K_{ave} (h^{-1})	0.104	0.095	0.150	0.085	0.149	0.109	0.115 (0.028)

Table 3. Pharmacokinetic parameters of indinavir in male Sprague–Dawley rats applying noncompartmental analysis (240-minute sampling period).

Parameter	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Mean (SD)
$AUC_{0\to240}$ (μM/min)	220.70	293.71	224.47	241.14	278.41	256.67	252.52 (29.37)
$AUC_{0\to\infty}$ (μM/min)	334.98	524.89	331.88	315.50	489.52	428.67	404.24 (89.81)
$AUMC_{0\to240}$ (μM/h^2)	4.32	4.31	3.46	3.99	4.88	4.10	4.18 (0.47)
$AUMC_{0\to\infty}$ (μM/h^2)	21.01	54.25	21.67	14.24	40.68	34.68	31.09 (14.94)
V_d (ss)(L/kg)	4.45	4.68	4.68	3.40	4.03	4.48	4.38 (0.45)
MRT (min)	225.77	372.07	235.08	162.51	299.14	291.21	264.30 (72.46)
Cl_{total} (mL/min/kg)	19.70	12.57	19.89	20.92	13.48	15.40	16.99 (3.62)
K_{ave} (h^{-1})	0.226	0.161	0.255	0.369	0.201	0.206	0.236 (0.072)

AUC and AUMC calculations and that these parameters are directly dependent on drug plasma concentration, we suggest that in cases similar to this study, the results of noncompartmental analysis are highly variable depending on the two main limiting factors: the duration of sampling time and the assay method.

A comparison between the pharmacokinetic parameters of indinavir determined from two different approaches is presented in Table 5. As shown, all the pharmacokinetic parameters are statistically different between the three groups of results ($P < 0.01$). Based on the Tukey's post hoc test, all the data set pairs were different from each other. This, we believe could be, in part, due to the sensitivity of the noncompartmental approach to the sampling period (see 420 versus 240 minutes, Table 5). The total indinavir systemic clearance calculated using the compartmental analysis (35.2 ± 5.1 mL/min/kg; Table 1) is in agreement with the reported hepatic clearance of the drug (~43 mL/min/kg)[13–16]. This means that the elimination of the drug in rats is predominantly hepatic, a finding that has been reported previously in rats[13,15,17] and humans[1,3]. However, our estimates for total drug systemic clearance values (12.0 ± 2.1 and 17.0 ± 3.6 mL/min/kg for 420- and 240-minute sampling times, respectively) obtained with the noncompartmental analysis (Tables 2 and 3) are much smaller than the reported hepatic clearance estimates (i.e., 43 mL/min/kg), which is basically impossible. The MRTs determined based on the noncompartmental analysis in this study (264.3 ± 72.5 and 544.2 ± 123.2 minutes for 240- and 420-minute sampling times, respectively) are much higher than the MRTs reported upon oral administration of even higher doses of the drug to rats[17]. These findings are unrealistic considering the basic theory of noncompartmental analysis[19,20]. However, the MRT calculated based on the compartmental analysis (23.7 ± 5.9 minutes) is more meaningful in relation to the reported elimination half-life of the drug (i.e., 28.5 ± 2.2 minutes)[13,14,16–18] as well as the oral MRT of indinavir in rats (i.e., 73 ± 61 minutes)[17]. The V_d (ss) values determined by both series of our noncompartmental calculations (4.38 ± 0.45 and 6.40 ± 0.99 L/kg for 240- and 420-minute sampling times, respectively) are significantly different from the values (0.842 ± 0.278 L/kg) determined using compartmental analysis ($P < 0.01$; Table 4). According to the basic theory of volume of distribution, the following rank order should be found between different volumes of distribution in a multicompartmental model: V_d (ext) > V_d (β) > V_d (ss) > V_c [21,25]. Although this ranking is evident in our compartmental analysis results (Table 1), the V_d (ss) values determined by noncompartmental analysis (Tables 2 and 3) are much higher than the other volumes of distribution estimated for the drug. Again, this is a result of the inclusion of the data of terminal, very slow elimination phase in the calculations of the noncompartmental analysis.

Collectively, these findings suggest that the application of noncompartmental analysis for drugs showing a very slow terminal concentration decline rate with a low drug plasma concentration can be questionable. This is mainly because of the dependency of this type of analysis to both the duration of sampling time and the sensitivity and specificity of the drug assay method. Based on the results from this study, we suggest the use of compartmental pharmacokinetic analysis with precise inspection of the kinetic phases considering the relative contribution of each phase in the overall pharmacokinetics of the drug.

Table 5. Comparative compartmental and noncompartmental pharmacokinetic parameters of indinavir in six male Sprague–Dawley rats upon IV administration of 5 mg/kg indinavir sulfate.

	Compartmental analysis	Noncompartmental analysis[b]	
Parameter[a]	420-minute period[b]	240-minute period	420-minute period
V_d (ss)(L/kg)	0.84 (0.28)	4.38 (0.45)	6.40 (0.99)
MRT (minutes)	23.72 (5.91)	264.30 (72.46)	544.24 (123.23)
Cl_{total} (mL/min/kg)	35.18 (5.15)	16.99 (3.62)	12.03 (2.09)
K_{ave} (h^{-1})	2.64 (0.56)	0.236 (0.072)	0.115 (0.028)
$AUC_{0\rightarrow\infty}$ (μM/min)	191.26 (30.78)	404.24 (89.81)	560.97 (86.01)

[a]Based on the Tukey's post hoc test, all of the parameters are significantly different between three groups of data ($P < 0.01$). [b]Mean (SD).

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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