

## Kinetic disposition of ursolic acid in rats

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### ABSTRACT

Benznidazole and nifurtimox are two drugs that are used to treat trypanosomiasis. Ursolic acid (UA) reportedly acts against trypomastigotes and intracellular amastigotes of *Trypanosoma cruzi*. Accordingly, it is expected to have therapeutic benefits in the treatment of trypanosomiasis. Therapeutic application of a compound requires the investigation of its pharmacokinetic properties in order to obtain relevant information to design the *in vivo* assays and dose regimen. Regarding this, the current study aimed to evaluate the pharmacokinetic profile of UA administered to rats at different doses and routes (i.e., 1 mg/kg intravenously and 20 and 50 mg/kg orally). According to the results, the oral bioavailability was significantly different between the two groups that orally received the UA doses of 20 mg/kg (2.8%) and 50 mg/kg (1.55 %). The result suggests the interference of the poor aqueous solubility of UA on its absorption process. The pharmacokinetic parameters related to the distribution and elimination were similar. Accordingly, it can be concluded that at this dose range, there is no saturation in this process rendering a linear the kinetics. The pharmacokinetic properties of UA were observed in this study indicated that the improvement of water solubility in this medicine through pharmacotechnical resources would be a great utility for its oral bioavailability and development of a product with the potential therapeutic application. The oral administration of this new pharmaceutical formulation should be investigated in future studies.

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### Introduction

Ursolic acid (UA) is a triterpene of the  $\alpha$ -amyrin type found in numerous plant species, such as *Eriobotrya japonica*, *Rosmarinus officinalis*, *Psidium guajaval*, and *Diospyros leucomelas* (1). In recent years, several investigations have indicated different pharmacological actions such as anti-inflammatory (2, 3), antitumoral (4), antiparasitic (5), and antimicrobial properties for this substance.

Ferreira et al. (5) carried out *in vitro* and *in vivo* studies of the trypanocidal activity and acute toxicity of UA. They performed the *in vitro* assay using trypomastigote forms of *Trypanosoma cruzi* (*T. cruzi*). Their results demonstrated that UA was active at 25.5  $\mu$ M (IC<sub>50</sub>) and provided 77% of trypomastigote lysis at 128  $\mu$ M. In addition, they conducted an *in vivo* assay to evaluate the trypanocidal activity in BALB/C albino mice intraperitoneally infected with trypomastigote forms of the Bolivia strain of *T. cruzi* and orally treated with 20 mg/kg/day UA for 20 days. This treatment was compared with benznidazole administered at a dose of 20 mg/kg/day for 20 days. The results of the mentioned study indicated that UA led to a greater reduction in the number of parasites at the parasitemic peak decreased by 60% in parasitemia. Additionally, all the animals

treated with this triterpene displayed increased survival time. The lethal oral dose of UA was determined in BALB/C albino mice in a single administration at a dose of 2.000 mg/kg with observation for 72 h. Their results demonstrated no mortality or any signs of toxicity; accordingly, they concluded that UA can be safely used on an experimental basis (5).

According to the World Health Organization, over 5.500.000 people are infected with *T. cruzi* in endemic regions. The countries which include the higher numbers of infected people are Argentina, Brazil, and Mexico (6). In recent years, the epidemiological patterns have changed due to the migration process. The report of American trypanosomiasis has pointed out an increase in infected people mainly in the east of Europe (7).

Two drugs that are available for the treatment of American trypanosomiasis include nifurtimox and benznidazole. However, the ineffectiveness of the current drug therapy, in addition to the safety issues with the patients, leads to the search for new therapeutic alternatives. The medications are produced either with the development of new molecules or the combination of drugs with new molecules to create synergism and improve the effectiveness of available drugs.

The potential therapeutic application of UA in this scenario requires the understanding of its kinetic behavior. Therefore, it is possible to adopt an adequate dose regimen for short- and long-term studies. A simple, reliable, and robust high-performance liquid chromatography (HPLC) bioanalytical method was developed for the quantification of UA in plasma and validated to evaluate this kinetic disposition.

In this study, the UA kinetics was analyzed through its intravenous and oral administration to Wistar rats at two different doses. The kinetic process was evaluated and pharmacokinetic parameters were calculated which can be the basis of exposure protocols for impact evaluation in future studies.

## Materials and methods

### Chemicals and reagents

The UA and diazepam, used as internal standard (IS), were purchased from Sigma-Aldrich® (St. Louis, MO, USA). Moreover, chloroform, trichloroacetic acid, Tween 80, dimethyl sulfoxide (DMSO), and ethanol were obtained from Synth® (Diadema, SP, Brazil). In addition, HPLC-grade methanol and acetonitrile were purchased from J.T. Baker® (Mexico City, Mexico). Xylazine and ketamine were obtained from Agener Uniao® (SP, Brazil), and heparin sodium was purchased from Blau Farmaceutica® (Cotia, SP, Brazil). Furthermore, the water was purified by a Milli-Q® system (Millipore).

### Bioanalytical Method for determination of ursolic acid in plasma samples

The ACQUITY UPLC H-Class PLUS System with ultraviolet-visible detector was used in this study. The chromatographic separation was performed on a Sun Fire-TM C8-Waters® (4.6×250 mm, 5 µm) column protected by Van Guard column with the same stationary phase. They were both placed into the column oven at 35°C. The mobile phase was an acetonitrile-water mixture (70:30 v/v) in isocratic mode with UV detection at 225 nm. The flow rate, sample injection volume, and run time were 0.5 mL/min, 2 µL, and 20 min, respectively. The ratio of the peak area of the analyte to that of the IS (diazepam in acetonitrile) was used for drug quantitation. All samples, quality controls, and calibration standards were treated prior to chromatographic analysis. To this end, 100 µL of plasma was added to 100 µL of 10% trichloroacetic acid and 20 µL of IS. This procedure was followed by 30 sec of the vortex (Phoenix Lufenco®, Brazil) and centrifugation (Hermle®, Germany) at 15.000 rpm at 4°C for 12 min.

Afterward, 500 µL of chloroform was added to the supernatant (150 µL), and the mixture was vortexed and centrifuged at 15.000 rpm for 15 min. In the next stage, 400 µL of the organic layer was evaporated to dryness under a vacuum condition (Genevac mini Vac Sample Concentrator Range®). The residue was resuspended in 100 µL mobile phase, and the solution was filtered in polytetrafluoroethylene (Analytica®, Brazil) filters of

0.22 µm directly into the injection vial of the chromatographic system. The bioanalytical method was validated according to the USA Food and Drug Administration (FDA) (8), Guidance for Industry, and (9) Brazilian Health Regulatory Agency (ANVISA) resolution 27/2012 (Agencia Nacional de Vigilância Sanitária, 2012). The calibration curve was linear ( $r^2 = 0.9999$ ,  $n = 8$ ) with a range of 0.05-2.5 µg/mL and a quantitation limit of 50 ng/mL. The inter- ( $n = 5$ ) and intraday ( $n = 10$ ) precisions of the coefficient of variation were obtained at  $\leq 10.2\%$  and  $\leq 12.3\%$  on the same day and between days, respectively, in terms of each quality control samples of 50, 150, 500, and 1500 µg/mL. In addition, the ranges of inter- and intraday accuracy (% bias) were estimated at -0.87-1.8 and -3.2-2.2 on the same day and between days, respectively, regarding each quality control sample.

### Experimental protocol

The pharmacokinetic assay was carried out using 15 male Wistar rats weighing 200-250 g. The animals were housed in polypropylene cages and kept under standard laboratory conditions ( $23 \pm 1^\circ\text{C}$ ) with food and water ad libitum. The animals were subjected to catheter insertion (intramedic polyethylene tubing, i.d. 0.28 mm/o.d. 0.61 mm; Becton, Dickinson and Company®, Sparks, MD, USA) in the femoral artery, venous blood sampling, and intravenous infusion 24 h before drug administration. The catheter was connected to PE-50 polyethylene tubing (CPL Medical's®, São Paulo, Brazil), which was exteriorized through the dorsal skin (10).

To this end, the animals were anesthetized with ketamine (7.3 mg/kg) and xylazine (5.5 mg/kg) through intraperitoneal injection. For intravenous (IV) UA administration (1 mg/kg,  $n = 5$ ), a mixture of DMSO and physiological saline solution was used at the ratio of 90:10. All IV administrations were performed over a short period of time (i.e., less than 30 sec). For oral UA administration (20 mg/kg,  $n=5$  and 50 mg/kg,  $n=5$ ), a mixture of 5% of ethanol, 2.5% Tween 80, 5% DMSO, and sufficient water was used as a vehicle. This preformulation has already been used previously to assess the toxicity of UA (11). The proportion of compounds used in the preformulation mentioned above did not exceed the limits recommended by Neervanan in order to avoid toxic effects (12). Blood samples (0.25 mL) were collected from the catheter previously inserted in the femoral artery in heparinized tubes after 0.08, 0.25, 0.5, 0.75, 1, 2, 3, 6, and 12 h.

The blood was centrifuged (Gilson, USA) at 3.500 rpm for 15 min to separate the plasma, which was then stored at  $-20^\circ\text{C}$  until analysis. The animals remained conscious during the drug administration and blood sample collection. They were deprived of food 12 h before drug administration. This protocol was approved by the Research Ethics Committee of the School of Pharmaceutical Sciences of São Paulo State University Araraquara, Brazil, (process 31/2015).

### Pharmacokinetic analysis

The kinetic disposition of UA was evaluated after the IV (1 mg/kg) and oral administration (20 and 50 mg/kg) of this drug to Wistar rats in a single dose. The elimination half-life ( $t_{1/2}$ ) was determined by the elimination phase of the graph of log plasma concentration versus time. The absorption half-life ( $t_{1/2a}$ ) was determined by the method of residuals. The elimination ( $K_{el}$ ) and absorption ( $K_a$ ) constants were calculated using the following formula:  $0.693/t_{1/2}$  or  $t_{1/2a}$ .

The  $K_a$  was used to calculate the mean absorption time (MAT) by the formula  $1/K_a$ . The area under the curve from 0 to the last quantifiable concentration ( $AUC_0-t$ ) was calculated by the trapezoidal method. Moreover, the area under the curve from 0 to infinity ( $AUC_0-\infty$ ) was calculated using the formula  $AUC_0-t + (C_n/k_{el})$ , where  $C_n$  was the last quantifiable plasma UA concentration.

Furthermore, the area under the moment curve (AUMC) was calculated by the statistical moment method and used to determine the mean transit time (MTT) =  $AUMC/AUC_0-\infty$ . The mean residence time (MRT) was calculated using the following equation:  $MRT = MTT - MAT$ .

The clearance (Cl) and the distribution volume ( $V_{area}$  or  $V_z$ ) were determined by two equations (i.e.,  $Cl = \text{dose}/AUC_0-\infty$  and  $V_z = Cl/k_{el}$ ) corrected by bioavailability for each group subjected to oral administration. In addition, the central volume of distribution ( $V_c$ ) was calculated as follows:  $V_c = \text{dose} \times F/B$  Where,  $B$  is the intercept of the y-axis of the elimination line.

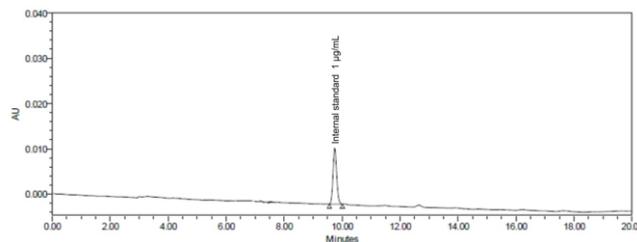
The distribution volume at steady-state ( $V_{ss}$ ) was calculated by  $V_{ss} = Cl \times MTT$ . The maximum plasma drug concentration ( $C_{max}$ ) was obtained directly from the experimental data, as was the time of the occurrence of  $C_{max}$  ( $t_{max}$ ). The  $F$  was evaluated by  $AUC_0-\infty$  oral x IV dose,  $AUC_0-\infty$  IV x oral dose. The described formulas were applied in Excel software for the calculation of pharmacokinetic parameters. The values were confirmed in the Phoenix® WinNonlin® software.

### Statistical analysis

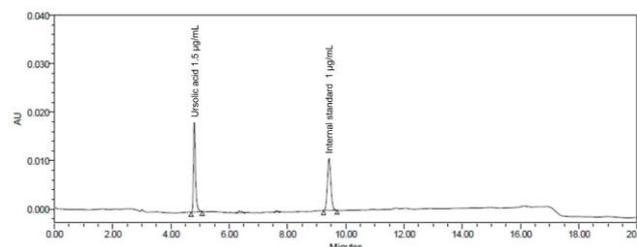
The data for pharmacokinetic parameters were expressed as mean with 95% confidence interval (95% CI). The groups were compared by the nonparametric Mann-Whitney U test (Graph Pad InStat software, version 3.06). Moreover, p-value less than 0.05 was considered statistically significant.

### Results

The chromatographic system presents an adequate chromatogram to the blank plasma and the plasma with UA (Figures 1 and 2). Therefore, this system facilitated the separation of the UA and IS at required levels for pharmacokinetic study. Table 1 demonstrates the data of the calibration curve with precision and accuracy. It can be observed that the relative standard deviation (RSD) values are within the acceptance criteria according to ANVISA guidelines. The calibration curve and linear regression were obtained through these data (Figure 3).



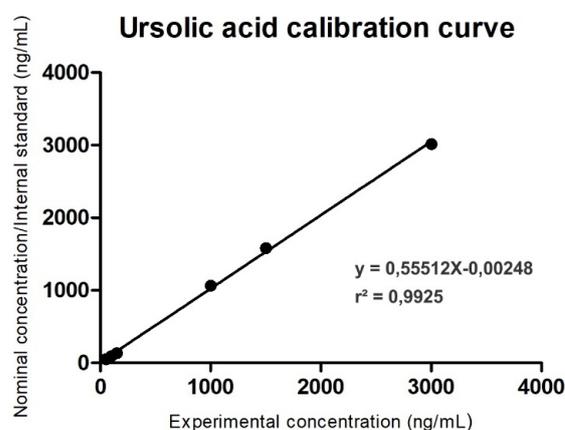
**Figure 1** Chromatogram of blank plasma with an internal standard (diazepam 1 µg/mL) at a wavelength of 225 nm and mobile phase: acetonitrile/water dilution of 70:30.



**Figure 2** Chromatogram of plasma with 1.5 µg/mL ursolic acid (retention time: 4.8 min) and internal standard (diazepam 1 µg/mL; retention time: 9.5 min) at a wavelength of 225 nm and mobile phase: acetonitrile/water dilution of 70:30.

**Table 1** Mean values of experimental concentrations generating the analytical curve and the precision expressed by the relative standard deviation and curve accuracy (n = 3).

Nominal (ng/mL)	50	100	150	1000	1500	3000
Experimental	51.23	95.69	134.6	1066.43	1581.74	3014
Relative standard deviation (%)	0.8	6.5	5.8	0.9	5	3.3
Accuracy	102.5	95.7	89.7	106.6	105.4	100.5



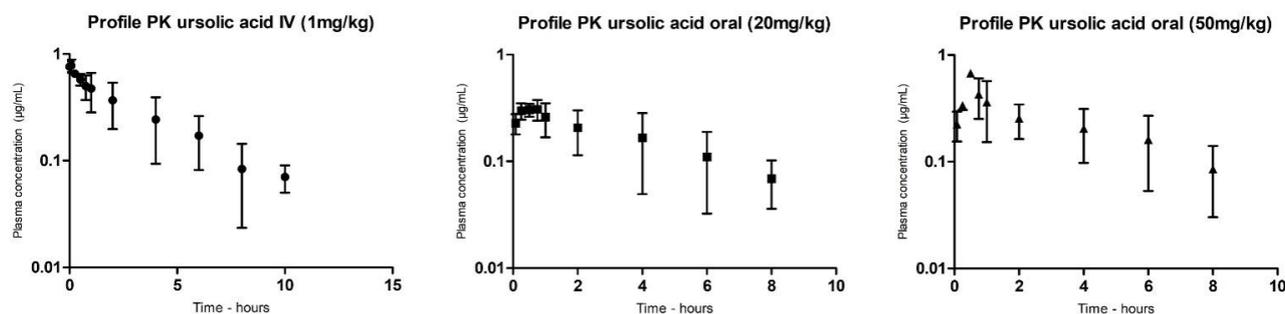
**Figure 3** Bioanalytical curve of ursolic acid in plasma (n = 3)

It is possible to verify that the correlation coefficient was higher than 0.9 in agreement with the ANVISA guidelines. Figure 4 and table 2 illustrate the PK profile of UA administered intravenously (1 mg/kg) and orally (20 and 50 mg/kg). Moreover, the pharmacokinetic parameters for all groups were tabulated, and the statistical comparison is displayed in table 3.

## Discussion

The results of the Mann-Whitney U test revealed no statistically significant difference between the groups receiving the compound orally at different doses in terms of the pharmacokinetic parameters of UA, except for bioavailability. The administration of a UA dose of

50 mg/kg provided more variable plasma levels with consequences on the oral variability of bioavailability for this group. The obtained maximum concentration was statistically different between the groups with a lower value in the group receiving 20 mg/kg, which was already expected (Table 2). In the current study, there were no significant differences between the groups in terms of the elimination constant ( $K_{el}$ ) and elimination half-life (elimination  $t_{1/2}$ ). This indicates that the route of administration and dose did not interfere with the elimination rate of the UA. These results demonstrate that the absorption process does not interfere with the elimination process.



**Figure 4** Plasma concentration versus time profile of ursolic acid administered to rats (male, 250 g, n = 5 each group) intravenously (1 mg/kg) and orally (20 and 50 mg/kg). (Data are expressed as mean±95%CI).

**Table 2** Plasma concentration of ursolic acid administered to Wistar rats (male, 250 g, n = 5 each group) intravenously (1mg/kg) and orally (20 and 50 mg/kg) (Data are expressed as mean±95%CI).

Time (plasma concentrations of ursolic acid)	IV (1 mg/kg) Cp (ug/mL)±CI95	Oral (20 mg/kg) Cp (ug/mL)-CI95	Oral (50 mg/kg) Cp (ug/mL)-CI95
0	0.76±0.09	-	-
0.083	0.82±0.08	0.10±0.04	0.13±0.08
0.25	0.66±0.04	0.23±0.08	0.22±0.14
0.5	0.60±0.06	0.30±0.08	0.34±0.08
0.75	0.55±0.10	0.30±0.06	0.38±0.05
1	0.56±0.09	0.31±0.08	0.46±0.23
2	0.44±0.08	0.26±0.10	0.41±0.08
3	-	0.21±0.11	0.29±0.14
4	0.31±0.1	0.17±0.11	0.23±0.14
6	0.17±0.07	0.11±0.07	0.18±0.15
8	0.09±0.05	0.07±0.03	0.09±0.08
10	0.06±0.02	0.05±0.01	0.05±0.03
12	0.05±0.02	-	-

There was no statistical difference between the oral groups;  $P < 0.01$ , Mann-Whitney U test

**Table 3** Pharmacokinetic parameters of ursolic acid administered to Wistar rats (male, 250 g, n=5 each group) intravenously (1mg/kg) and orally (20 and 50 mg/kg) (Data are expressed as mean±95%CI).

Parameter	IV group (1 mg/kg) Mean±95%CI	Oral group (20 mg/kg) Mean±95%CI	Oral group (50 mg/kg) Mean±95%CI
kel (h <sup>-1</sup> )	0.28±0.09	0.24±0.03	0.26±0.04
t <sub>1/2</sub> (h)	2.61±0.74	2.88±0.03	2.7±0.46
C <sub>p0</sub> or C <sub>max</sub> (ug/ml)	0.76±0.12	0.33±0.08 <sup>a</sup>	0.50±0.17 <sup>a,b</sup>
t <sub>max</sub> (h)	-	1.05±0.7	1.18±0.88
ASC <sub>0-t</sub> (ug/ml.h)	2.83±0.95	1.44±0.65 <sup>a</sup>	2.08±1.14 <sup>a</sup>
ASC <sub>0-inf</sub> (ug/ml.h)	2.99±1.06	1.68±0.65 <sup>a</sup>	2.33±1.20 <sup>a</sup>
Cl (ml/h/kg)	357.69±136.51	357.14±121.39	368.29±226.10
V <sub>d<sub>c</sub></sub> (ml/kg)	1329.22±201.62	1690.73±347.13	1519.62±445.62
V <sub>d<sub>z</sub></sub> (ml/kg)	1279.46±204.26	1488.35±574.10	1426.55±801.24
V <sub>d<sub>ss</sub></sub> (ml/kg)	1390.14±265.54	1641.44±445.31	1544.48±643.50
MTT (h)	4.05±0.97	4.63±0.71	4.24±0.5
MRT (h)	4.05±0.97	3.7±0.80	2.98±0.38
Ka (h <sup>-1</sup> )	-	4.12±2.4	1.96±1.27
t <sub>1/2a</sub> (h)	-	0.27±0.23	0.39±0.21
MAT(h)	-	0.39±0.33	0.57±0.30
F (%)	-	2.80±0.82	1.55±0.8 <sup>b</sup>

IV: intravenous administration group

Oral: oral administration groups

AUC 0-t: area under the concentration-time curve from 0 to the last quantifiable concentration

AUC 0-∞: area under the concentration-time curve from 0 to the last quantifiable concentration from 0 extrapolated to infinity

Kel: elimination constant

t<sub>1/2</sub>: elimination half-life

Cl: clearance

V<sub>z</sub>: distribution volume at pseudo-equilibriumV<sub>c</sub>: central volume of distributionC<sub>max</sub>: maximum plasma concentrationT<sub>max</sub>: time of occurrence of C<sub>max</sub>

Ka: absorption constant

t<sub>1/2a</sub>: absorption half-life

MAT: mean absorption time

MTT: mean transit time

MRT: mean residence time

<sup>a</sup>: statistical difference (IV group); <sup>b</sup>: statistical difference (oral group) 20 mg/kg; P<0.01, Mann-Whitney U testV<sub>d<sub>ss</sub></sub>: distribution volume at steady-state

F : bioavailability

In the dose range studied, the distribution and elimination phases of the UA presented linear kinetics. The elimination t<sub>1/2</sub> is an important parameter in the determination of the dosing schedule in a multiple-dose regimen. The relationship between elimination t<sub>1/2</sub> and dosing interval (τ) will determine the degree of fluctuation in plasma concentrations (ρ), accumulation of the drug in the body (R), and time required to reach steady-state levels. The UA elimination half-life value of 2-3 h can be considered relatively short. For the new molecules under study, > 6 h was considered an optimal value for the half-life parameter (13). Drugs with terminal half-lives shorter than 12 h have an accumulation rate of less than 1.3 and plasma oscillation greater than 2.0 when given once daily. The UA elimination half-life value between 2 and 3 h may limit the range of administrations if the oscillation of plasma concentrations allowed to

achieve the desired effect is restricted (i.e., if the therapeutic window is narrow). It is important to emphasize the importance of studying the relationship between plasma concentration and its effects on the definition of this therapeutic window. The elimination half-life is a hybrid pharmacokinetic parameter, which undergoes interference from two primary pharmacokinetic parameters, including CL and distribution volume. The CL is a very important concept in pharmacokinetics because it describes the removal of the drug from the body either by metabolism or excretion in an unchanged form. However, this parameter is not always well understood because it indirectly measures the drug removal, unlike the elimination half-life. The CL is expressed as the volume of biological fluid that is free of drug per unit time not as the amount of drug eliminated per unit time.

The CL can be influenced by blood flow, cardiac output, and enzyme activity regarding metabolizing organs. Its magnitude is assessed by the cardiac output of the animal model studied. The maximum value of CL is limited by the blood flow, which delivers the drug to the metabolizing and excretory organs. The liver and kidneys are the main effectors of these processes, and the blood flow to these organs corresponds to approximately half of the cardiac output of the organism. In this way, high CL is considered a value equal to or greater than half of the cardiac output. Furthermore, when the CL value of a drug is higher than the blood flow to the major metabolizing and excretory organs, other organs and processes participate in the elimination of the compound.

The cardiac output can be calculated by an allometric equation ( $CO [ml/kg/min]=180 \times BW^{0.19}$ ), where BW is the body weight in kilogram. For Wistar rats with a mean weight of 0.25 kg, half the cardiac output value is 117.12 ml/kg/min. The CL of UA obtained in Wistar rats was about 6 ml/kg/min, which may be considered a low value for the animal model once that the body extraction rate ( $E_{body}$ ) is equal to 0.05.

A low rate of extraction is desirable for the oral administration of drugs. This low extraction increases the residence time of the compound in the organism allowing a greater interval of administration. The CL parameter is useful to compute the dosing rate required to yield the desired steady-state target plasma concentration. Moreover, the average steady-state concentration of a drug was calculated during multiple-dose regimens.

If the amount of drug excretion in the unchanged form is known, it is possible to calculate the renal CL ( $CL_r$ ). Moreover, it leads to the conceptualization of the principal mechanism of the elimination of the compound whether it is an unchanged excretion or metabolism. This knowledge is useful in the evaluation of situations regarding the risk of overdosage or toxic effects in renal or hepatic impairment.

In the UA case, the short elimination half-life is a limiting factor in terms of the selection of dose interval. As it is stated earlier, the elimination  $t_{1/2}$  is a pharmacokinetic parameter that relates to CL and distribution volume or volume of distribution. The distribution volume can be considered the size of the compartment possessing the total amount of drug in the body which is in dynamic equilibrium with the drug in the blood.

With regard to the blood volume of 0.07 L/kg, the higher values of distribution volume suggest the extravascular accumulation of the drug, as is the case of UA, the distribution volume values of which were around 1 L/kg. This result is in line with the findings obtained by Quina et al. where they suggested an accumulation of UA in the organs with great blood perfusion, such as the lung, liver, and heart (14).

When the plasma concentration is decreased by the elimination process, the corresponding amount of drug present in the extravascular compartment is shifted and the dynamic equilibrium is maintained. Therefore, the

concentration of drug maintained in the extravascular compartment is constantly modified with changes in intravascular concentrations.

Although the extent of distribution seems to be the most important aspect of this process; at first sight, the rate of distribution occurrence is also of great importance. This is because the rate of distribution may have a significant impact on the timing of the pharmacological effect at the desired intensity, especially if the major site of the drug action is in the extravascular compartment.

The entire process of drug distribution can occur rapidly or slowly, involving more than one subsequent step. The achieved plasma concentration following intravenous administration results from the administered dose and drug distribution in the body. This distribution can be amplified as the drug reaches the other sites in its affinity; however, it increases in sites in which the arrival rate is lower.

Accordingly, there is an initial distribution volume ( $V_c$  or  $V_i$ ), which is the result of a rapid distribution to the so-called "central" compartments. In addition, there is a volume of distribution after the occurrence of the steady-state ( $V_{ss}$ ) or pseudo-equilibrium state ( $V_{area}$ ) among the various pharmacokinetic processes. When the drug has a rapid distribution profile, the values of  $V_c$ ,  $V_{ss}$ , and  $V_{area}$  are similar. On the other hand, when the distribution occurs at different rates for different organs and tissues, the values of  $V_c$ ,  $V_{ss}$ , and  $V_{area}$  are different, and  $V_c$  would be always smaller. The values of the three types of volume distribution calculated for UA did not present significant statistical differences (Table 3). Accordingly, it can be concluded that the extravascular accumulation of UA occurs rapidly after absorption.

As previously mentioned, the rate of distribution may interfere with the time of the occurrence of the maximal effect, particularly if the site of action is in the extravascular compartment. If the distribution occurs rapidly, the maximum effect will also take place rapidly and probably simultaneously at maximal blood concentrations, as is the case of UA.

The plasma profile obtained after extravascular drug administration is strongly influenced by the absorption process. It is important to quantify the magnitude of the absorption as well as quantify the rate of absorption to understand this process. The absorption parameters related to the rate, either directly ( $K_a$ ,  $t_{1/2a}$ , and MAT) or indirectly ( $C_{max}$  and  $T_{max}$ ), were evaluated between the groups that received two different oral doses. These parameters, except for  $C_{max}$ , did not show statistical differences. The values for  $K_a$ ,  $t_{1/2a}$ , and MAT revealed that the UA absorption was rapid and linear for this dose range (i.e., 20-50mg/kg). The difference observed in  $C_{max}$  was expected because this parameter was also influenced by the dose level and was higher for the 50 mg/kg group. The absorption parameters related to extension, bioavailability, and area under the curve indicated low efficiency, with absolute bioavailability values lower than 3%. A relevant aspect observed in the evaluation of the extent of absorption is the significant difference between the groups receiving different doses by the oral route.

The group receiving the highest dose (50 mg/kg) had lower bioavailability for UA than the group administered the lowest dose (20 mg/kg).

The drug absorption in the gastrointestinal tract can be influenced by several drug characteristics, such as solubility and permeability, as well as physiologic factors, including pH, gastric emptying time, and intestinal motility. A plausible explanation for the lower efficiency of absorption at the higher dose is the low solubility of UA in water, which hindered the formation of a molecular dispersion in the intestinal lumen. When a compound has very low aqueous solubility, it is erratically and incompletely absorbed. This results from the inability to dissolve in the gastrointestinal tract following oral administration as the case of UA. This feature has also limited the use of this compound as medicines, foods, and cosmetics (15).

Another important observation is that poor dissolution is responsible for the high variability in drug absorption and represents a major problem in drug design. As stated earlier, the dose of 50 mg/kg provided more variable plasma levels and more variability in the oral bioavailability of UA. The obtained knowledge about the phenomena involved in drug absorption and the characteristics that influence this process allows defining the best routes of administration. In addition, it facilitates planning for pharmaceutical systems or formulations leading to the improvement of the undesirable characteristics of the drug that can cause failures to reach the levels necessary for the pharmacological effect.

Oral administration is preferred due to its convenience and safety with relatively low production costs. However, the delivery of the drug to its intended target site depends on the efficiency of intestinal absorption and the pre-systemic drug metabolism. As the results revealed, the obtained oral bioavailability was low and variable. This demonstrates the need for new formulations that can improve the oral absorption of the compound.

## Conclusion

According to the general pharmacokinetic characteristics observed in this study, it is possible to consider a critical point for the development of a pharmaceutical product. The characteristics suitable for the continuity of the therapeutic studies of UA include the incorporation of the compound into a pharmaceutical system that improves the solubility supporting the doses required for the desired effects. The incorporation of the compound in this system is accompanied by an increase in the extent of oral uptake and a decrease in the variability of this process. Therefore, subsequent activity studies are more reproducible. The incorporation of UA into stealth liposomes may be an interesting route since this type of vehicle has been shown to extend blood circulation time. At the same time, it reduces the mononuclear phagocyte system uptake (16) that is appropriate for acting against *T. cruzi*.

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## Conflicts of interest

The authors confirm that this article has no conflicts of interest.

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