Saudi Pharmaceutical Journal (2017) **25**, 413–418

King Saud University

Saudi Pharmaceutical Journal

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ORIGINAL ARTICLE

Effect of nitazoxanide on albendazole pharmacokinetics in cerebrospinal fluid and plasma in rats

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Received 24 November 2015; accepted 24 September 2016
Available online 30 September 2016

KEYWORDS

Albendazole;
Albendazole sulfoxide;
Nitazoxanide;
Tizoxanide;
Cerebrospinal fluid;
Pharmacokinetics

Abstract *Background:* Although albendazole is the drug-of-choice for the treatment of neurocysticercosis, its efficacy is limited due to its low bioavailability. An alternative for optimizing pharmacological treatment is through drug combinations. *In vitro* studies have shown that nitazoxanide and tizoxanide (the active metabolite of nitazoxanide) exhibit cysticidal activity and that the combination of tizoxanide with albendazole sulfoxide (the active metabolite of albendazole) produced an additive effect. *Objectives:* (1) To assess the concentration profile of tizoxanide in plasma and in cerebrospinal fluid; and (2) to evaluate the influence of nitazoxanide on the pharmacokinetics of albendazole in plasma and in cerebrospinal fluid. *Methods:* Two different studies were conducted. In study 1, 10 male Sprague-Dawley rats received a single oral dose of 7.5 mg/kg of nitazoxanide and serial blood and cerebrospinal fluid samples were collected over a period of 4 h. In study 2, 38 healthy male Sprague-Dawley rats were randomly divided into two groups: one of these received a single dose of albendazole (15 mg/kg) and, in the other group, albendazole (15 mg/kg) was co-administered with nitazoxanide (7.5 mg/kg). Plasma and cerebrospinal fluid samples were collected from 0 to 16 h after administration. Albendazole sulfoxide and tizoxanide levels were assayed by

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jsps.2016.09.005

using HPLC or LC/MS techniques. *Results:* In study 1, tizoxanide reached a maximum plasma concentration of 244.42 ± 31.98 ng/mL at 0.25 h; however, in cerebrospinal fluid, this could be detected only at 0.5 h, and levels were below the quantification limit (10 ng/mL). These data indicate low permeation of tizoxanide into the blood brain barrier. In study 2, Cmax, the area under the curve, and the mean residence time of albendazole sulfoxide in plasma and cerebrospinal fluid were not affected by co-administration with nitazoxanide. *Conclusion:* The results of the present study indicate that in rats at the applied doses, tizoxanide does not permeate into the cerebrospinal fluid. Furthermore, nitazoxanide does not appear to alter significantly the pharmacokinetics of albendazole in plasma or in cerebrospinal fluid.

1. Introduction

Neurocysticercosis (NCC) is the most important parasitic infection of the nervous system caused by the larval form of the tapeworm *Taenia solium*. The infection is acquired when a human ingests oncospheres of *Taenia*, which permeate into the small blood vessels and migrate to muscles, brain, and other tissues (Nash and Garcia, 2011). NCC is the major cause of seizures and a public health problem in many countries of the Indian subcontinent and Latin America, including Mexico (Fleury et al., 2012; Ndimubanzi et al., 2010). Pharmacological treatment of NCC is based on two cysticidal drugs: praziquantel (PZQ) and albendazole (ABZ). Given its efficacy, to date ABZ is preferred over PZQ (Del Brutto et al., 2006). ABZ binds to the β-subunit of tubulin, inhibiting its polymerization, thus preventing microtubule formation (Lacey, 1990; Martin, 1997).

ABZ is rapidly and extensively metabolized to albendazole sulfoxide (ABZ-SO), an active metabolite. In humans, the metabolite permeates into the cerebrospinal fluid (CSF); however, due to the low solubility of ABZ, its bioavailability is low and variable, and therefore plasma and CSF levels of ABZ-SO are also highly variable among individuals (Jung-Cook, 2012).

In order to increase ABZ bioavailability, different strategies have been proposed during the last 10 years: for example, administration of ABZ with a fatty meal (Mares et al., 2005), use of surfactants in solid dispersions (Castro et al., 2013), the development of binary and ternary formulation systems with cyclodextrins, as well as the use of water soluble polymers or hydroxyacids (Casulli et al., 2006; Kalaiselvan et al., 2006, 2007; Palomares-Alonso et al., 2010). Recently, a novel intranasal microemulsion has been evaluated for the delivery of ABZ-SO to the brain (Shinde et al., 2015). Despite the positive results reported, none of them is available in the market.

The combination with existing drugs offers another alternative for increasing ABZ effectiveness. Little research has been performed in this area, and the majority of the studies are related to the ABZ–PZQ combination. For example, an *in vitro* additive interaction between PZQ and ABZ-SO was reported by our group (Palomares et al., 2006). It has also been demonstrated that plasma levels of ABZ-SO increased when the combined treatment was administered in patients with NCC (Garcia et al., 2011). Recently, the same authors documented that this combination was more effective than ABZ alone in patients with parenchymal NCC (Garcia et al., 2016).

Another drug that has demonstrated activity against different nematodes and trematodes is nitazoxanide (NTZ) (Anderson and Curran, 2007; van den Enden, 2009). This drug is used as a broad-spectrum antiparasitic drug in adults and children in many areas of the world (Somvanshi et al., 2014). Also, this drug has demonstrated cysticidal efficacy against *Taenia crassiceps* cysts (Palomares-Alonso et al., 2007). After its oral administration, NTZ is partially absorbed from the gastrointestinal tract and is rapidly hydrolyzed by plasma esterases into its desacetyl derivative, tizoxanide (desacetylnitazoxanide, TZO) which is the active metabolite (Stockis et al., 1996). Although its mechanism of action is not well known, it has been postulated that in helminths, NTZ is a non-competitive inhibitor of the pyruvate ferredoxin oxidoreductase enzyme, altering anaerobic metabolism (Romero et al., 1997; Walker et al., 2004).

Considering that the mechanisms of action of NTZ and ABZ are different, this combination has been evaluated for the treatment of echinococcosis and cysticercosis. Thus, Stettler et al. (2004) found that the use of NTZ in combination with ABZ improved the ABZ pharmacokinetics as well as efficacy in the echinococcosis murine model. In the case of cysticercosis, Palomares-Alonso et al. (2007) reported that the combination of NTZ and ABZ, as well as ABZ-SO and TZO, resulted in an additive effect against *Taenia crassiceps* cysts *in vitro*.

Taking into account that one of the major obstacles to successful pharmacological management of NCC is the presence of the blood brain barrier (BBB) (Nau et al., 2010), the present study attempted to determine whether TZO is capable of crossing the BBB and to evaluate the influence of NTZ on the pharmacokinetics of ABZ in plasma and in CSF.

2. Materials and methods

2.1. Chemicals and reagents

ABZ-SO and TZO standards used for the analytical methodology were synthesized by Drs. Rafael Castillo-Bocanegra and Alicia Hernández Campos at the Facultad de Química, UNAM, Mexico. Chemical identity was confirmed by NMR, MS analysis, and melting point determination. ABZ, mebendazole (MBZ), and nifuroxazide (NFZ), used as internal standards (IS) for plasma ABZ-SO and plasma TZO analysis, respectively, were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Methanol, acetonitrile, ether, dichloromethane, and chloroform were of HPLC grade (Mallinckrodt

Co., St. Louis, MO, USA). Formic acid (Sigma-Aldrich Co., St. Louis, MO, USA) was of analytical reagent-grade. Water was obtained from a Milli-Q Water System (Millipore Corporation, Bedford, MA, USA).

For the pharmacokinetic studies, ABZ suspension (Zentel®, 400 mg/10 mL; Glaxo SmithKline) and NTZ suspension (Paramix®, 100 mg/5 mL; Liomont Laboratories) were used.

2.2. Animal experiments

2.2.1. Animal care and surgery

Male Sprague-Dawley rats weighing 305 ± 15 g were purchased from Harlan (Harlan, Mexico City, Mexico). The animals were housed one per cage and were maintained at a temperature of 18–23 °C and at 40–70% relative humidity under 12-h light:12-h dark cycles. Food and water were freely available. The study protocol complied with the Guide to the Care and Use of Experimental Animals and was approved by the Animal Care Committee of the Instituto Nacional de Neurología y Neurocirugía (INNN) (Mexico City, Mexico).

For the cannulation procedure, each animal was anesthetized by an intraperitoneal injection of Ketamine (50 mg/kg) and Xylazine (10 mg/kg).

2.2.1.1. CSF collection. Animals were cannulated in cisterna magna using the technique reported by Consiglio and Lucion (2000). Briefly, rats were anesthetized as described previously and the surgical region was shaved. The animal was immobilized in a stereotaxic apparatus and an incision was made on the top of the head. The stereotaxic parameters for cannulating the rat were the following: antero-posterior (AP) = −2.7 mm (posterior to the interaural line); vertical (V) = −6.2 mm (below the dura mater); lateral = 0, and superior incisive = −3.2 mm under the interaural line. Once the system was localized, trepanation was performed. A stainless steel tube 1 cm in length with 23-G thickness with a rounded tip was inserted with an inclination of 31°, 2 mm in depth, and fixed with dental cement. Rats were allowed to recover from anesthesia for a period of 48 h.

2.2.1.2. Plasma collection. Rats were cannulated in the lateral tail vein under anesthesia following a procedure that was previously described by de Jong et al. (2001). An incision was made 3 cm from the tail base and the vein was exposed; a previously heparinized polyethylene cannula (ID 0.023 in and OD 0.038 in) (Becton Dickinson, Franklin Lakes, NJ, USA), was introduced and fixed with a 2–0 surgical suture. The animals were left to recover for a period of 1–3 h prior to the pharmacokinetic study. During the study, the animals received water *ad libitum*.

2.2.2. Pharmacokinetic studies

The study was divided into two different experiments. In the first experiment, the objective was to assess TZO permeation through the BBB. In this study, plasma and CSF pharmacokinetics was evaluated. In the second experiment, the pharmacokinetic effect of concomitant administration of ABZ and NTZ in plasma and the CSF pharmacokinetic profile of ABZ-SO were studied. For NTZ, a 7.5 mg/kg dose was selected according to the dose used in humans for the treatment of diarrhea associated with *Giardia lamblia* or *Cryptosporidium parvum*; in addition, this dose has been proposed for the treatment of mixed parasitic infections (protozoa and helminths) (Romero et al., 1997; Fox and Saravolatz, 2005). For ABZ, we selected the dose employed for NCC treatment in humans (15 mg/kg) (Jung-Cook, 2012).

2.2.2.1. Study 1. Pharmacokinetic study of TZO. Ten rats were administered orally with a dose of 7.5 mg/kg of NTZ. Plasma and CSF samples (three blood samples of 0.6 mL, and three CSF samples of 20 µL per animal) were obtained at 0, 15 min, 30 min, 1 h, 2 h, and 4 h after drug administration (with five replicates per sampling time). Blood samples were collected in heparinized tubes and centrifuged at 3,000 rpm for 10 min. Plasma and CSF samples were stored at −70 °C until analysis.

2.2.2.2. Study 2. Effect of NTZ on ABZ pharmacokinetics in plasma and in CSF. Rats were randomly assigned to two treatment groups (n = 19 each): In Group I, the animals received a single dose (15 mg/kg) of ABZ, and in group II, the animals received ABZ and NTZ in combination (15 mg/kg + 7.5 mg/kg, respectively). Drugs were orally administered. Blood and CSF samples (three blood samples and three CSF samples per animal) were collected at 0, 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, 10 h, 12 h, 14 h, and 16 h after drug administration (with five replicates per sampling time).

2.3. Drug assays

Complete validation of the analytical procedures for drug quantitation was performed before the pharmacokinetic studies. For preparation of calibration curves as well as quality controls and blank samples, rat plasma and artificial CSF were used. Artificial CSF was prepared with NaCl (125 mM), KCl (2.5 mM), $MgCl_2$ (1.18 mM), and $CaCl_2$ (1.26 mM) dissolved in 1.5 L of phosphate-buffered saline (PBS) solution pH 4 (Na_2HPO_4 2.97 mM, and NaH_2PO_4 1.06 mM).

2.3.1. Plasma and CSF quantitation of TZO

The chromatographic system (Waters, Milford, MA, USA) consisted of a Model 2690 Waters Alliance HPLC pump, with a Model 2996 photodiode array detector (set at 416 nm) and a Model 2690 Waters Alliance autosampler (set at 15 °C) equipped with a 100-µL loop. Chromatographic separation was performed using an X-Terra C_{18} analytical column (150 × 4.6 mm, 5 µm; Waters) attached to a pre-column (Phenomenex C_{18} ODS). The column oven was maintained at 25 °C. A ternary gradient was used for the chromatographic separation. The initial mobile phase was composed of KH_2PO_4 50 mM buffer (pH 5.7):methanol:acetonitrile (90:5:5, v/v/v). A linear gradient was achieved varying the mobile-phase composition during 23 min up to the final mixture, KH_2PO_4 50 mM buffer (pH 5.7):methanol:acetonitrile (10:45:45, v/v/v). For plasma analysis, a previously published technique was employed (Ruiz-Olmedo et al., 2009). Plasma samples (200 µL) were spiked with 100 µL of NFZ (6,000 ng/mL) as IS. After the addition of 200 µL of acetonitrile for protein precipitation, samples were vortex-mixed for 2 min and centrifuged at 15,000 rpm for 20 min. Supernatant was obtained and 100 µL was injected into the chromatographic system. CSF samples (20 µL) were analyzed without any pre-

treatment steps by using the external calibration method in artificial CSF. Calibration curves for both fluids were constructed by least-squares linear regression with correlation coefficients of $\geqslant$0.998. For plasma, the method was linear within a range of 20–1280 ng/mL; the method was precise (relative standard deviation, RSD, $<$8%) and accurate (absolute deviation, $<$8%). In the case of CSF, linearity was assessed within the range of 10–320 ng/mL. For precision, the RSD was $<$15% and the relative error (RE) of accuracy was less than 15%.

2.3.2. ABZ-SO assay in plasma

Plasma levels of ABZ-SO were determined by a liquid chromatography-mass spectrometry (LC-MS) method. The LC-MS system consisted of a Finnigan Surveyor LC pump Plus and a Finnigan Surveyor autosampler Plus coupled to a Finnigan LCQ Advantage Max ionic trap mass spectrometer (Thermo Electron Corporation, San Jose, CA, USA). Chromatographic separation was performed using a Gemini C_{18} analytical column (150 × 4.6 mm, 5 µm, Phenomenex, Torrance, CA, USA) protected with a pre-column (Phenomenex C_{18} ODS). The mobile phase was composed of methanol:-formic acid 20 mM (70:30, v/v) at a 0.7 mL/min flow rate with a split ratio of 1:3. Compounds eluting from the column were analyzed in the positive ion mode, with 3.5-kV capillary voltage. Nitrogen was employed as sheath gas (23 arb) and auxiliary gas (4 arb). The heated capillary temperature was set at 290 °C. Quantitation was performed using selected reaction monitoring (SRM) of the transitions of m/z 282 → 240 for ABZ-SO and of m/z 296 → 264 for MBZ (IS). The collision energies of 25 and 32 eV were used for ABZ-SO and IS, respectively.

A volume of 200 µL of plasma sample was transferred into an assay tube and spiked with 100 µL of 400 ng/mL IS in methanol. After the addition of 4.5 mL of ether-dichlorome thane-chloroform (60:30:10, v/v/v), samples were vortex-mixed for 5 min and centrifuged for 30 min at 1408g. The supernatant was transferred to a clean glass-assay tube and evaporated to dryness in a water bath at 45 °C with a nitrogen stream. The residue was reconstituted with 160 µL of methanol:water (70:30 v/v), and a 25 µL aliquot was injected into the chromatographic system. Linearity for ABZ-SO in plasma was assessed within the range of 20–3000 ng/mL. Calibration curves were plotted using ABZ-SO area/IS area vs. concentration and analyzed by 1/x weighted least-squares linear regression. Correlation coefficient was $\geqslant$0.998. The method was precise (RSD, $<$8%) and accurate (RE, $<$8%).

2.3.3. ABZ-SO assay in CSF

ABZ-SO in CSF levels was determined by HPLC. A 20 µL aliquot of CSF was injected directly into the chromatographic system, which consisted of a Waters Alliance 2690 HPLC pump (Waters) coupled to a photodiode array detector (Model 2296 Waters) set at 293 nm and a Model 2690 Waters Alliance autosampler. Separation was performed using an Atlantis T3 analytical column (150 × 1 mm, 3.5 µm; Waters) protected with a pre-column (Phenomenex C_{18} 4 mm × 3 mm; Phenomenex). The autosampler and column oven were set at 15 and 30 °C, respectively. For mobile phase, a gradient was employed as depicted in Table 1. The flow rate was set at 0.06 mL/min. The method was linear within the range of 10–1500 ng/mL with correlation coefficients of $\geqslant$0.99. The method was precise (RSD, $<$1%) and accurate (RE, $<$14%).

Table 1 Gradient for ABZ-SO analysis in CSF samples.

Time (min)	Component (%)		
	Water	Methanol	Acetonitrile
0	95	2.5	2.5
15	40	30	30
16	40	30	30
25	10	45	45
35	10	45	45

2.4. Pharmacokinetic and statistical analysis

Plasma and CSF concentration-time data were analyzed by a non-compartmental model using WinNonLin ver. 5.0 software (Pharsight Corp., Mountain View, CA, USA). Peak plasma concentration (Cmax), time to reach peak plasma concentration (Tmax), area under the curve (AUC) to the last measurable plasma concentration point ($AUC_{0\rightarrow t}$), elimination half-life ($t_{1/2}$), and mean residence time (MRT) were calculated. Results are expressed as mean ± standard error of the mean (SEM). Statistical analysis was assessed by the Student t test. Differences were considered statistically significant at $p < 0.05$.

3. Results and discussion

3.1. Study 1

When the pharmacokinetics of NTZ was evaluated, we found that, as expected, TZO was the only measurable species in plasma (Fig. 1). TZO reached a maximum plasma concentration (Cmax) of 244.42 ± 31.98 ng/mL at 0.25 h. This low plasma concentration could be related to incomplete absorption of NTZ from the gastrointestinal tract (Huang et al., 2015).

With respect to the pharmacokinetic parameters of NTZ, we found that plasma elimination half-life was short (1.47 h) and similar to that reported in humans (1.53 h) (Stockis et al., 1996). Moreover, MRT was also short (1.27 ± 0.17 h).

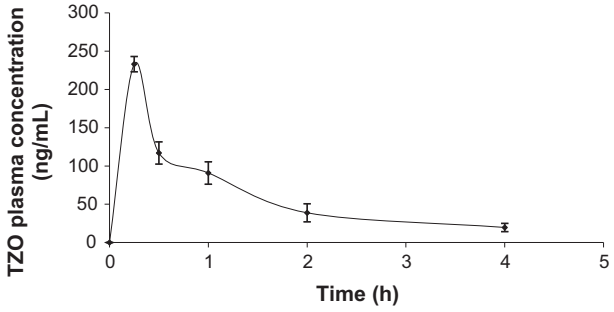

Figure 1 Tizoxanide plasma concentration time profile after an oral dose of 7.5 mg/kg nitazoxanide. Each point represents the mean ± SD, n = 5.

Cmax and AUC showed a low interindividual variability (% RSD: 13 and 26, respectively).

When CSF samples were analyzed, TZO was undetectable at nearly all sampling times. The metabolite was detected only at 0.5 h; however, TZO levels were below the quantification limit (10 ng/mL). To date, to our knowledge, brain permeation of TZO has not been reported. Our results suggest low access of the metabolite into the brain. The low permeation could be related to the low levels of TZO in plasma or its short elimination half-life. Considering that only the unbound plasma fraction can freely penetrate to CSF, another possibility could be associated with TZO's high protein binding (98%) (Zhao et al., 2010).

3.2. Study 2

The results of our study revealed that in rat, ABZ was rapidly metabolized to its main active metabolite: ABZ-SO. As in humans, ABZ-SO also crossed the BBB and reached the CSF. In this fluid, the metabolite was detected from the first sampling time and during the entire interval (16 h). Fig. 2 presents ABZ-SO profiles in plasma and in CSF, and Table 2 summarizes the main plasma and CSF pharmacokinetic parameters.

When ABZ was administered alone, a great inter-individual variability in plasma levels was found (Fig. 2). The absorption process was slow: ABZ-SO maximum concentration was reached at 6.8 h. Tmax and MRT values were similar to those reported by Merino et al. (2003) after the administration of a 10.6 mg/kg dose of ABZ in the same species. When ABZ-SO pharmacokinetic parameters in plasma and CSF were compared, we found that Tmax and MRT were similar.

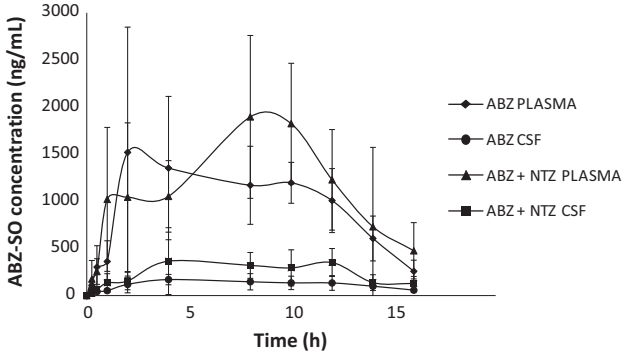

Figure 2 Albendazole sulfoxide plasma and CSF concentration time profile after an oral dose of 15 mg/kg albendazole and after an oral dose of 15 mg/kg albendazole and 7.5 mg/kg nitazoxanide. Each point represents the mean ± SD, $n = 5$.

When ABZ was administered in combination with NTZ we found that, although plasma levels at 8 and 10 h after administration were slightly higher in comparison with the administration of ABZ alone, the differences were not significant. In the case of CSF, ABZ-SO levels were also higher in the combined treatment without statistical significance. Likewise, no statistical differences were found in MRT in plasma and in CSF between both treatments. These results indicate that under the study conditions, NTZ did not markedly modify ABZ pharmacokinetics.

In a previous study in mice, it was found that the combined treatment of NTZ and ABZ increased ABZ-SO serum levels during the 4–8 h interval after administration. The authors suggested that the uptake of one drug affected the absorption of the other drug (Stettler et al., 2004).

Taking these results into account, we expected an increase in ABZ-SO levels in plasma and also in CSF. The lack of pharmacokinetic interaction found in the present study could be associated with the following factors which might influence the solubility and bioavailability of one or both drugs: (a) The doses administered, as the doses of ABZ and NTZ employed in the present study were 15 mg/kg plus 7.5 mg/kg, while the doses used by Stettler et al. were 33 mg/kg plus 150 mg/kg; (b) The dosage form. While in our study commercial suspensions of ABZ and NTZ were employed, Stettler et al. used the pure drugs suspended in 0.5% carboxymethylcellulose, and (c) the differences in the animals' species used. It has been reported that the fluid content in the gastrointestinal tract in mice is smaller than that found in rats (McConnel et al., 2008).

Few studies have been performed to evaluate pharmacokinetic interactions of NTZ with other anthelminthic drugs. In 2015, Ceballos et al. (2015) evaluated the combination of flubendazole and NTZ in mice. As in our study, authors found that NTZ did not significantly affect the efficacy and pharmacokinetics of flubendazole.

4. Conclusion

Since tizoxanide (the active metabolite of nitazoxanide) did not cross the blood brain barrier and that plasma and cerebrospinal fluid levels of albendazole sulfoxide (the active metabolite of albendazole) were also not markedly affected by tizoxanide, the combination of albendazole and nitazoxanide would not be a suitable alternative for neurocysticercosis treatment.

Table 2 Non-compartmental pharmacokinetic parameters for ABZ-SO in rat plasma and CSF after oral administration of 15 mg/kg ABZ and 15 mg/kg plus 7.5 mg/kg NTZ (mean ± SD, $n = 5$).

Parameter	ABZ		ABZ plus NTZ	
	Plasma	CSF	Plasma	CSF
Cmax (ng/mL)	1729.30 ± 1190.76	170.40 ± 79.22	2124.35 ± 871.18	466.94 ± 312.37
Tmax (h)	6.80 ± 3.63	8.40 ± 3.85	9.60 ± 0.89	8.40 ± 2.97
AUC (ng h/mL)	16371.40 ± 7125.28	1885.23 ± 832.47	19301.31 ± 8690.05	3892.51 ± 2178.37
$t_{1/2}$ (h)	2.53 ± 0.65	4.16 ± 1.27	2.25 ± 0.55	3.73 ± 1.66
MRT (h)	7.40 ± 0.62	8.36 ± 0.89	8.15 ± 0.39	7.67 ± 0.67

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