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Original Article

Bioavailability, tissue distribution, and excretion characteristics of the novel carbonic anhydrase inhibitor tolsultazolamide in rats

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Aim: Tolsultazolamide, a novel carbonic anhydrase inhibitor, is designed for the prophylaxis and treatment of acute mountain sickness. The aim of this study was to investigate the pharmacokinetics, tissue distribution, and excretion characteristics of tolsultazolamide and the sex difference in pharmacokinetics in rats.

Methods: For pharmacokinetic study, rats were intravenously injected tolsultazolamide at 1 and 2 mg/kg or orally administered tolsultazolamide at 20, 40, or 80 mg/kg) in a pharmacokinetic study. The concentrations of tolsultazolamide in plasma were determined with high-performance liquid chromatography, with a liquid–liquid extraction. For tissue distribution study, tolsultazolamide (80 mg/kg) was orally administered to overnight fasted rats (six per group and three per sex). Samples were collected from the brain, heart, lung, liver, spleen, muscle, kidney, stomach, fat, intestines, pancreas and sexual gland. For excretion study, tolsultazolamide (40 mg/kg) was orally administered to 6 rats (three per sex). The urine, feces, and bile samples were collected at 24, 48, and72 h.

Results: After its intravenous administration, tolsultazolamide was rapidly eliminated from the plasma, with $T_{1/2}$ of about 60–90 min. The AUC_{0-t} and the initial concentration (C0) values were proportional to the intravenous doses. After its oral administration, tolsultazolamide showed dose-independent pharmacokinetic characteristics, with T_{max} and $T_{1/2}$ of approximately 2 h and 5–7 h, respectively, and good oral absolute bioavailability of about 60%. Tolsultazolamide was distributed widely in various tissues. The highest tolsultazolamide levels were detected in the stomach, intestine, spleen, lung, and kidney. Total excretion of unchanged tolsultazolamide in the urine, feces, and bile was less than 2%. The C_{max} and AUC of tolsultazolamide were significantly higher in female rats than those in male rats. Clearance and volume of distribution were greater in male rats than those in female rats. The oral absolute bioavailability was also significantly different between female rats (about 83%) and male rats (about 37%).

Conclusion: Tolsultazolamide was well absorbed and widely distributed in the rat, and very little of the unchanged form was excreted. Sex had a significant effect on the pharmacokinetics of tolsultazolamide.

Keywords: carbonic anhydrase inhibitors; tolsultazolamide; pharmacokinetics; tissue distribution; excretion; sex difference

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Introduction

Acute mountain sickness (AMS) is a common disease occurring at high altitudes. It is caused by hypoxia and characterized by headache, weakness, fatigue, nausea, insomnia, and depressed appetite. Although AMS does not seem serious, if untreated it can progress to the life-threatening conditions of high-altitude pulmonary edema and high-altitude cerebral edema[1–4]. Because acute exposure to high altitudes is increasingly common for both work- and travel-related reasons, AMS is an issue of national and international concern.

The carbonic anhydrase inhibitor acetazolamide is now widely accepted as an effective drug for the prophylaxis and treatment of AMS, but the high incidence of its adverse effects, including paresthesia, diuresis, and dysgeusia, limits its application[5–8]. Therefore, an effective and safe drug for the prevention and treatment of AMS is urgently required. Tolsultazolamide (Figure 1A), a new derivative of acetazolamide (Figure 1B), is a novel carbonic anhydrase inhibitor discovered by our research team. The results of animal experiments have indicated that it improves the endurance of mice under hypoxic conditions and may function more effectively than acetazolamide[9, 10].

Pharmacokinetic studies play a very important role in drug discovery and development, not only to support toxicological

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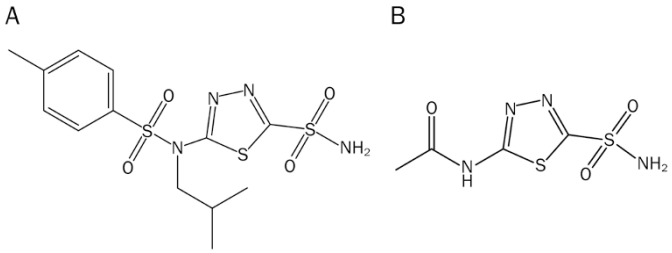

Figure 1. Structures of tolsultazolamide (A) and acetazolamide (B).

and clinical studies but also to optimize drug candidates[11]. Therefore, the objective of the current study was to examine the pharmacokinetics, tissue distribution, and excretion characteristics of tolsultazolamide in rats. The influence of sex on the pharmacokinetics of tolsultazolamide was also studied.

Materials and methods

Reagents

Tolsultazolamide (purity >98.0%) was prepared by Academy of Military Medical Sciences (Beijing, China). Phenacetin (internal standard, IS, purity >98.0%) was purchased from Sigma Chemical Co (St Louis, CA, USA). Methanol of HPLC grade was obtained from Anhui Fulltime Specialized Solvents & Reagents Co Ltd (Anhui, China). Ultra-pure water was prepared using a Milli-Q water purification system (Millipore, USA). Other reagents and solvents were of analytical grade.

Instrumentation and analytical conditions

The RP-HPLC analysis was performed on a Dionex HPLC system equipped with an ultraviolet detector (Dionex Corp, USA). Chromatographic separation was achieved using a Zorbax Eclipse Plus C_{18} column (4.6 mm×250 mm, 5 μm) coupled with a pre-column Zorbax Eclipse Plus C_{18} (4.6 mm×12.5 mm, 5 μm) at temperature of 25 °C. The mobile phase consisted of water (contained 0.1% acetic acid) and methanol (42:58, *v/v*), at a flow rate of 1.0 mL/min. The sample injection volume was 20 μL, and the detection wavelength was 285 nm.

Animals

Adult wistar rats [200±20 g, certificate No: SCXK-(Military) 2007-004] of both sexes were obtained from the Experimental Animal Center of the Academy of Military Medical Sciences (AMMS, Beijing, China). All animal experiments were performed in accordance with the Animal Care and Use Guidelines set by AMMS Animal Care and Use Committee.

Pharmacokinetic study

Oral administration

Three groups of rats (six per group and three per sex) were orally administered tolsultazolamide (suspended in 0.5% CMC-Na) at a dose of 20, 40, or 80 mg/kg. Blood samples (about 0.4 mL at each time point) were collected through the ophthalmic venous plexus at 20 min, and 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 12, 24, and 32 h after the oral administration of the drug. All blood samples were immediately centrifuged at 1500×*g* for 10 min and the plasmas were frozen at -20 °C until analysis.

Intravenous administration

Two groups of rats (six per group and three per sex) were intravenously injected with tolsultazolamide (dissolved with 1% DMSO and 1% cremophor) at a dose of 1 or 2 mg/kg. Blood samples (about 0.4 mL at each time point) were collected through the ophthalmic venous plexus at 2, 5, 10, 15, 30, 45, 60, and 120 min after the intravenous injection of the drug. All blood samples were immediately centrifuged at 1500×*g* for 10 min and the plasmas were frozen at -20 °C until analysis.

Tissue distribution study

Tolsultazolamide (80 mg/kg) was orally administered to four groups of overnight fasted rats (six per group and three per sex). Samples were collected at 0.5, 2, 8, and 24 h post-dosing from the brain, heart, lung, liver, spleen, muscle, kidney, stomach, fat, intestines, pancreas and sexual gland, thoroughly rinsed with physiological saline, and then blotted dry with filter paper and stored at -20 °C.

Excretion study

Urine and feces excretion

After oral administration of tolsultazolamide (40 mg/kg), six rats (three per sex) were immediately placed in metabolism cages, and samples of urine and feces were collected separately and quantitatively at the following intervals: 0–24 h, 24–48 h and 48–72 h. The volumes of the urine samples were recorded. Fecal samples were dried and weighed, and then homogenized with 10-fold volumes of water for assay.

Bile excretion

Six rats (three per sex) were anesthetized with 10% chloral hydrate (0.3 mL/100 g) and bile duct cannulation was performed to allow bile collection. Bile samples were collected at intervals of 0–6 h, 6–12 h, 12–18 h, and 18–24 h after the oral administration of tolsultazolamide (40 mg/kg). The volumes of the bile samples were measured for analysis.

Sample pretreatment

For pharmacokinetic study, 150 μL plasma mixed with 20 μL IS (10 μg/mL phenacetin methanol solutions) were vortexed for 30 s and then extracted by 1 mL dichloromethane for 3 min. The substratum was transferred to a clean tube after centrifuged at 1500×*g* for 5 min, and then dried by N_2 at 40 °C. The residue was reconstituted with 50 μL mobile phase and centrifuged at 9000×*g* for 5 min, and 20 μL of the supernatant was injected into the HPLC system for analysis.

For tissue distribution study, small slices of tissues were individually homogenized with 4-fold volumes of physiological saline. A 300 μL homogenate spiked with 20 μL IS and 100 μL 0.1 mol/L HCl were vortexed for 30 s and then extracted by 1 mL dichloromethane for 3 min. Other operations were the same as pharmacokinetic study.

For excretion study, 150 μL (urine or bile) or 100 μL feces

homogenate of each interval sample was extracted by 1 mL dichloromethane for 3 min. Other operations were the same as pharmacokinetic study.

Pharmacokinetic and statistical analysis

Pharmacokinetic parameters of tolsultazolamide were calculated using non-compartmental methods (WinNolin ver 5.2, Pharsight Corp, Mountain View, CA, USA). The maximum plasma concentrations (C_{max}) and the time to reach the maximum concentrations (T_{max}) were obtained directly from the observed data. The elimination rate constant (k_e) was determined by linear regression of the terminal portion of plasma concentration-time data. The elimination half-life ($T_{1/2}$) was calculated as $0.693/k_e$. The area under plasma concentration-time curve (AUC_{0-t}) to the last measurable plasma concentration (C_t) was estimated by the linear trapezoidal rule. The area under the plasma concentration-time curve to time infinity ($AUC_{0-\infty}$) was calculated as $AUC_{0-\infty}=AUC_{0-t}+C_t/k_e$. The mean residence time (MRT) was calculated as $AUMC_{0-\infty}/AUC_{0-\infty}$. The total body clearance (CL) was calculated as $Dose/AUC_{0-\infty}$. The apparent volume of distribution (V_d) was calculated as CL/k_e [12, 13].

Dose-proportionality was evaluated by comparison of the dose-normalized AUC_{0-t} and C_{max} across different dosage levels and linear regression analysis[12, 13]. Data were expressed as mean±standard deviation (SD). The significance of difference was assessed by Student's *t*-test between two groups and one-way variance analysis (ANOVA) between three groups. A *P*-value<0.05 was considered statistically significant.

Results

Method validation

Under the chromatographic conditions, tolsultazolamide and IS were resolved well and were free from endogenous interference. The retention times of tolsultazolamide and IS were approximately 15 min and 5.5 min, respectively (Figure 2). The standard curves showed excellent linearity, with correlation coefficients greater than 0.99. The lower limit was defined as the lowest concentration at which both the precision and accuracy were less than or equal to 20% (Table S1 and S2). The inter- and intra-day precision (RSD) were below 15%, and the accuracy was in the range of 85%–115%. The extraction recoveries of both tolsultazolamide and IS were greater than 70%. The stability of tolsultazolamide was assessed during all the storage steps of the experiment, including (a) in the plasma samples at -20 °C for 1 week, (b) in the plasma samples after two thaw cycles, and (c) in the extracted samples for 24 h at room temperature. It indicate that tolsultazolamide was stable under all the conditions as described above.

Pharmacokinetic study

Pharmacokinetic study after intravenous administration

The plasma concentration–time curves and the pharmacokinetic parameters of tolsultazolamide after its intravenous administration (1 or 2 mg/kg) are presented in Figure 3A and Table 1, respectively. After its intravenous administration, tolsultazolamide was rapidly eliminated from the plasma, with $T_{1/2}$ of about 60–90 min. The AUC_{0-t} and the initial concentration (C_0) values were proportional to the intravenous doses, with no statistical differences of the dose-normalized AUC_{0-t} (AUC_{0-t}/D) and C_0 (C_0/D) between the two doses ($P>0.05$). However, other pharmacokinetic parameters such as $T_{1/2}$, CL, and V_d were independent of the intravenous doses.

Table 1. Pharmacokinetic parameters of tolsultazolamide in rats after intravenous administration at two different doses. *n*=6. Mean±SD. $^bP<0.05$, $^cP<0.01$, comparison between the two groups.

Parameters	1 mg/kg	2 mg/kg
k_e (1/min)	0.010±0.006	0.014±0.004
$T_{1/2}$ (min)	85.2±33.4	54.0±20.2
C_0 (μg/mL)	1.922±0.293	3.340±1.430^b
AUC_{0-t} (min·μg/mL)	14.169±2.543	27.299±8.016^c
$AUC_{0-\infty}$ (min·μg/mL)	17.992±3.835	31.013±8.120^c
V_d (L/kg)	6.716±2.208	5.400±2.556
CL (mL·min^{-1}·kg^{-1})	57.803±12.708	67.876±16.051

Pharmacokinetic study after oral administration

As shown in Table 2 and Figure 3B, after oral administration of tolsultazolamide at doses from 20 to 80 mg/kg, T_{max}, and

Table 2. Pharmacokinetic parameters of tolsultazolamide in rats after oral administration at three different doses. *n*=6. Mean±SD. $^cP<0.01$ among the three groups.

Parameters	20 mg/kg	40 mg/kg	80 mg/kg
k_e (1/h)	0.122±0.059	0.115±0.036	0.154±0.046
$T_{1/2}$ (h)	6.8±3.1	6.6±2.4	4.8±1.3
T_{max} (h)	2.2±0.5	2.1±0.6	1.8±0.5
C_{max} (μg/mL)	0.523±0.213	0.710±0.245	1.626±0.266^c
AUC_{0-t} (h·μg/mL)	2.730±1.290	5.580±1.839	11.438±1.637^c
$AUC_{0-\infty}$ (h·μg/mL)	3.893±2.024	6.004±1.796	12.278±2.533^c
V_d/F (L/kg)	60.019±37.779	71.526±45.825	45.925±12.909
CL/F (L·h^{-1}·kg^{-1})	6.444±3.352	7.174±2.106	6.740±1.324
MRT (h)	4.8±0.3	8.4±0.9	7.5±1.3

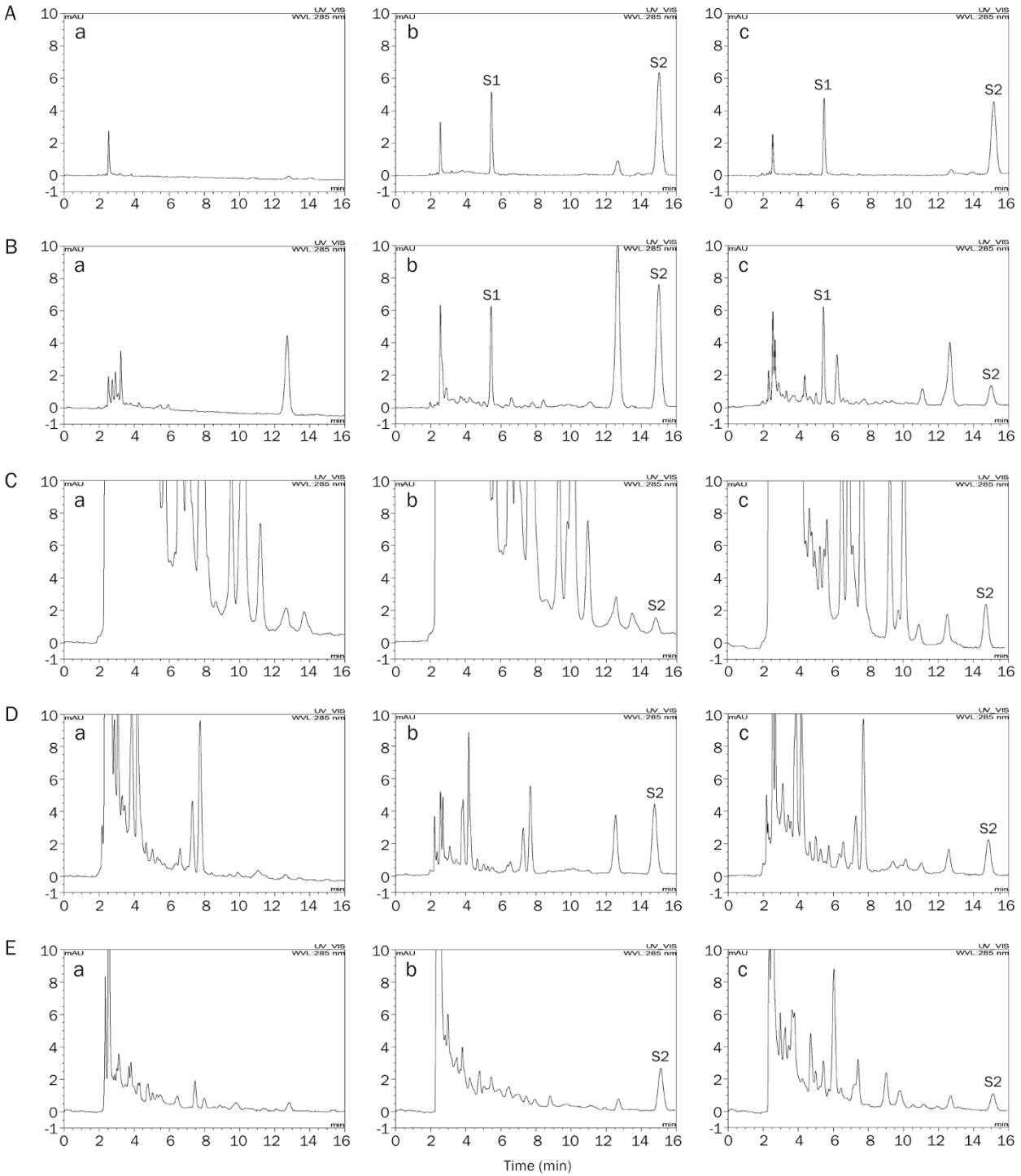

Figure 2. The HPLC chromatograms of tolsultazolamide in rat biological samples. (A-a) blank plasma; (A-b) blank plasma spiked with tolsultazolamide and IS; (A-c) actual plasma sample; (B-a) blank liver homogenate; (B-b) blank liver homogenate spiked with tolsultazolamide and IS; (B-c) actual liver homogenate sample; (C-a) blank urine; (C-b) blank urine spiked with tolsultazolamide; (C-c) actual urine sample; (D-a) blank feces homogenate; (D-b) blank feces homogenate spiked with tolsultazolamide; (D-c) actual feces homogenate sample; (E-a) blank bile; (E-b) blank bile spiked with tolsultazolamide; (E-c) actual bile sample. (S1, IS; S2, tolsultazolamide).

$T_{1/2}$ were both dose independent and were approximately 2 h and 5–7 h, respectively. AUC_{0-t} and dose, as well as C_{max} and dose, showed good linear relationships, with correlation coefficients (r^2) of 1 and 0.985, respectively. In addition, the dose-normalized AUC_{0-t} (AUC_{0-t}/D) and C_{max} (C_{max}/D) were not significantly different among the three doses analyzed by

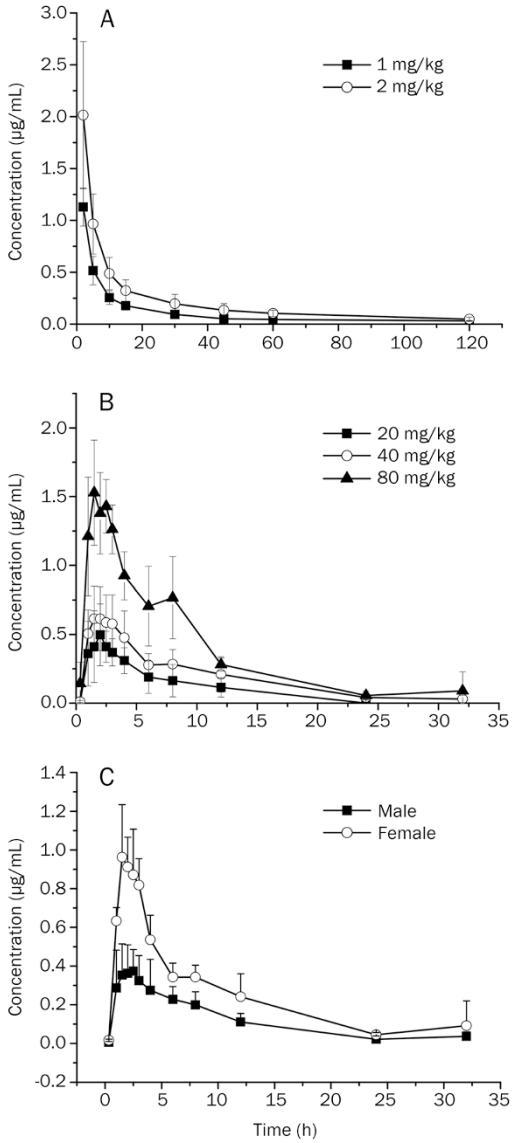

Figure 3. Plasma concentration-time profiles of tolsultazolamide in rats (mean±SD). (A) Pharmacokinetic profiles of 1 and 2 mg/kg tolsultazolamide administered intravenously (n=6); (B) Pharmacokinetic profiles of 20, 40, and 80 mg/kg tolsultazolamide administered orally (n=6); (C) Pharmacokinetic profiles of male and female rats after the oral administration of 40 mg/kg tolsultazolamide (n=5 for each sex).

ANOVA (P>0.05).

Oral absolute bioavailability of tolsultazolamide

The absolute bioavailability F(%) of tolsultazolamide was estimated with the following equation[14]: $F(\%)=[(AUC_{oral}\times Dose_{iv})/(AUC_{iv}\times Dose_{oral})]\times 100\%$, where AUC_{oral} and AUC_{iv} are the areas under the concentration–time curves and $Dose_{iv}$ and $Dose_{oral}$ represent the doses intravenously and orally administered, respectively. The mean oral absolute bioavailability of tolsultazolamide administered at 20, 40, and 80 mg/kg was 58.91%±1.56%, 60.02%±1.59%, and 61.70%±1.63%, respectively, and bioavailability was thus dose independent (P>0.05). The final average oral absolute bioavailability was 60.27%±0.04%.

Sex differences in the pharmacokinetics of orally administered tolsultazolamide

During our study of the oral pharmacokinetic properties of tolsultazolamide, we observed that the plasma concentrations of tolsultazolamide differed greatly between the male and female rats. Therefore, a further experiment was performed to investigate the influence of sex on the pharmacokinetics of tolsultazolamide. The pharmacokinetic parameters of orally administered tolsultazolamide (40 mg/kg) in each sex are given in Table 3, and the plasma concentration–time curves are shown in Figure 3C. Significant sex differences were observed in C_{max} and AUC, which were about twofold higher in the female rats than those in the male rats. In contrast, the V_d/F and CL/F values in the female rats were significantly lower than those in the male rats. In addition, the oral absolute bioavailability was also significantly different between female rats (about 83%) and male rats (about 37%). However, there was no significant difference between the sexes in T_{max} or $T_{1/2}$.

Table 3. Pharmacokinetic parameters of orally administered tolsultazolamide in different sexes. n=5. Mean±SD. ^bP<0.05, ^cP<0.01, comparison between the two groups.

Parameter	Male	Female
k_e (1/h)	0.097±0.050	0.119±0.021
$T_{1/2}$ (h)	8.6±3.8	6.0±1.1
T_{max} (h)	2.9±1.8	1.8±0.4
C_{max} (μg/mL)	0.421±0.130	1.045±0.178^c
AUC_{0-t} (h·μg/mL)	3.452±1.010	7.123±0.962^c
$AUC_{0-\infty}$ (h·μg/mL)	4.285±0.752	8.062±1.373^c
V_d/F (L/kg)	122.762±61.235	44.410±13.932^b
CL/F ($L\cdot h^{-1}\cdot kg^{-1}$)	9.606±1.938	5.074±0.832^c
MRT (h)	8.6±2.0	8.3±1.0

F: ~37% for male, ~83% for female.

Tissue distribution

The tissue distribution of tolsultazolamide was investigated following a single oral dose of 80 mg/kg. The concentrations of tolsultazolamide in the various tissues were measured at 0.5, 2, 8, and 24 h after its administration (Figure 4). Tolsultazolamide was identified in all the tissues tested 0.5 h after its administration, suggesting its rapid and extensive distribution. In most tissues, the concentration of tolsultazolamide reached a peak at 2 h and was still elevated 8 h after drug administration. The highest tolsultazolamide levels were detected in the stomach, intestine, spleen, lung, and kidney. By 24 h, less than 50% of the maximum tolsultazolamide remained in most tissues.

Excretion

The excretion-time profile data for tolsultazolamide in the

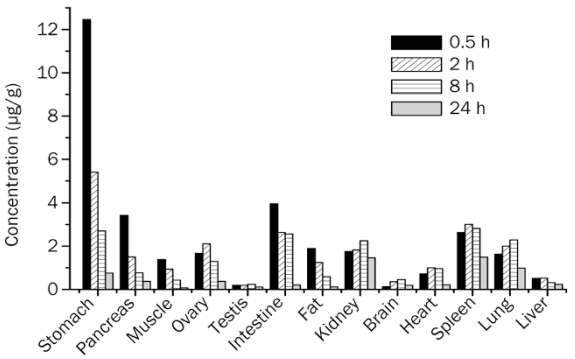

Figure 4. Tissue distribution of tolsultazolamide in rats after the oral administration of 80 mg/kg (mean±SD. *n*=6).

urine, feces, and bile after a single oral dose of 40 mg/kg are shown in Table 4. The average cumulative excretion of tolsultazolamide was 0.016%±0.019% in the urine and 1.115%±0.506% in the feces within 72 h, and 0.015%±0.007% in the bile within 24 h.

Table 4. Excretion of tolsultazolamide from urine, feces and bile in rats after oral administration. *n*=6. Mean±SD.

Parameter	Urine (%)	Feces (%)	Bile (%)
0–24 h	0.007±0.010	1.032±0.556	0.015±0.007
24–48 h	0.006±0.005	0.078±0.127	–
48–72 h	0.003±0.004	0.005±0.006	–
Total	0.016±0.019	1.115±0.506	0.015±0.007

Discussion

In this study, we first evaluated the pharmacokinetics, tissue distribution, and excretion properties of a novel carbonic anhydrase inhibitor, tolsultazolamide, in the rat. The results of this study should provide a meaningful basis for the use of tolsultazolamide as a new drug for the clinical prevention and treatment of AMS.

A rapid and sensitive high-performance liquid chromatography method, with simple liquid-liquid extraction, was established and validated for the quantitative analysis of tolsultazolamide in various biological samples. After its oral administration, both AUC_{0-t} and C_{max} for tolsultazolamide showed perfect linear relationships with the oral dose, and there were no significant differences of the dose-normalized AUC_{0-t} (AUC_{0-t}/D) and C_{max} (C_{max}/D) among the three doses (20, 40, and 80 mg/kg), whereas other pharmacokinetics parameters such as $T_{1/2}$, CL, and V_d were dose independent. These findings support linear rather than non-linear pharmacokinetic profiles of tolsultazolamide in rats within the dose range studied. The value of CL/F was larger than 100 $mL \cdot min^{-1} \cdot kg^{-1}$, which was about twofold of the hepatic blood flow rate (about 55.2 $mL \cdot min^{-1} \cdot kg^{-1}$)[15], suggesting a rapid clearance of tolsultazolamide from the rat body. The value of V_d/F was higher than 45 000 mL/kg, which was much larger than the total blood volume (about 54.0 mL/kg) and the total body water in rat (about 671.0 mL/kg)[15], demonstrating a wide distribution of tolsultazolamide into the extravascular systems. The average oral absolute bioavailability of tolsultazolamide in the rat was about 60%, which demonstrated that tolsultazolamide is absorbed well in rats.

The tissue distribution of a drug is vital when investigating its major target sites and interpreting its disposition *in vivo*[16]. In our tissue distribution study, tolsultazolamide presented an extensive distribution profile and was detected in all the tissues examined, which were consistent with the results of V_d/F in the pharmacokinetic study. After its oral administration, the concentrations of tolsultazolamide peaked at 2 h in most tissues and declined dramatically by 24 h, indicating that there was no apparent accumulation of tolsultazolamide in tissues. The AUC_{0-24} in tissues was in descending order of stomach, spleen, kidney, intestine, lung, ovary, pancreas, plasma, heart, fat, muscle, brain, liver and testis (data not shown). The AUC_{0-24} of brain was about 44% of that in plasma, suggesting that the lipid solubility enabled tolsultazolamide to cross the blood-brain barrier. Furthermore, the relatively high distributions of tolsultazolamide in kidney and lung may contribute to its use for the prevention and treatment of AMS, for the kidney is regarded as the major target organ of acetazolamide in the prophylaxis and treatment of AMS and the lung is the major pathological organ in AMS[17, 18].

In our excretion experiments, we estimated the unchanged fraction of tolsultazolamide in the urine, feces, and bile. Only a small proportion (less than 2%) of tolsultazolamide was excreted intact, demonstrating the extensive metabolism of this drug after its oral administration in rats. Further studies are required to investigate the pathways of tolsultazolamide metabolism and to clarify whether tolsultazolamide itself or its metabolites make the major contribution to its significant pharmacological effects.

In recent years, the importance of sex differences in drug studies has been recognized[19–21], and consideration of these differences increases the safety and efficacy of drug-based therapies. In the present study, we found significant sex-based differences in the pharmacokinetics of orally administered tolsultazolamide, in that the concentrations of tolsultazolamide were almost higher in female rats than those in male rats at each time point. The C_{max} and AUC values in female rats were both about twofold higher than those in male rats, whereas the V_d/F and CL/F values in female rats were significantly lower than those in male rats ($P<0.05$). However, we did not observe significant differences in AUC values between female and male rats after intravenous administration of tolsultazolamide, so the oral absolute bioavailability of female rats was higher than that of male rats (about 83% and 37%, respectively), which would also cause the sex differences in CL/F and V_d/F after oral administration of tolsultazolamide. These results suggest that the observed sex differences in response to orally administered tolsultazolamide were probably caused

by the difference in oral absolute bioavailability between female and male rats. In addition, many other mechanisms could also underlie the sex-based differences, including physiological factors such as body weight, organ size, glomerular filtration, and bowel motility, and molecular factors, such as specific transporters and drug-metabolizing enzymes[20, 21]. In this study, the concentrations of tolsultazolamide in the kidneys also differed between the sexes, and were almost twofold higher in the female rats than those in the male rats at all the time points examined (data not shown). Therefore, further studies are required to explore the exact reasons for the sex-based differences in the pharmacokinetics of tolsultazolamide in rats and to investigate whether sex significantly influences the pharmacokinetics of tolsultazolamide in humans.

Tolsultazolamide is a derivative of acetazolamide, so it is very meaningful to compare its pharmacokinetics, tissue distribution, and excretion characteristics with acetazolamide. The T_{max} and $T_{1/2}$ of tolsultazolamide (about 2 h and 5–7 h, respectively) were very close to those of acetazolamide (about 1–3 h and 6 h, respectively). Moreover, both of them were well absorbed following oral administration, and widely distributed to body tissues. However, only a small proportion (less than 2%) of tolsultazolamide was excreted intact, which is very different from acetazolamide (most excreted unchanged in urine)[22].

Taken together, the achieved pharmacokinetics, tissue distribution and excretion results of tolsultazolamide may be useful for better understanding of the pharmacodynamics of tolsultazolamide and its mechanism of action, such as when and where it can be effective, and these results will also provide important information for evaluation of the safety of tolsultazolamide. Furthermore, these data will provide theoretical basis for designing drug treatment regiments of the clinical trials.

However, there were also some limitations of our study. In the current study, we did not study the effects of acute exposure to high altitude on the pharmacokinetics of tolsultazolamide. It is known that immediate exposure to high altitude will induce some physiologic changes, such as the redistribution of blood flow to the most metabolically active systems and the changes of the content of protein and red blood cell, and these physiologic changes may alter drug pharmacokinetics that, in turn, might require modifications in dosage regimens to maintain efficacy or prevent toxicity. Previous study has reported an increased clearance of acetazolamide after acute and chronic exposure to 4360 m[23]. Therefore, further studies are required to investigate the high-altitude pharmacokinetics characteristics of tolsultazolamide. In addition, the major metabolic products and pathways of tolsultazolamide were not investigated in the present study, which also need to be explored in the future studies.

Conclusions

This is the first study to evaluate the pharmacokinetics, tissue distribution, and excretion characteristics of tolsultazolamide in rats. Following its oral administration, tolsultazolamide was rapidly absorbed and showed relatively high bioavailability. The tissue distribution of tolsultazolamide was established rapidly and was widespread, but the total recovery of unchanged tolsultazolamide in the urine, feces, and bile was very low, indicating the extensive metabolism of tolsultazolamide. Sex also significantly affected the pharmacokinetics of orally administered tolsultazolamide. These results provide reliable scientific data, which helps to design safe and effective treatment regimens for the clinical administration of the drug.

Supplementary Information

Supplementary information was available on the Acta Pharmacologica Sinica's website.

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Author contribution

Jin-da WANG, Yong-ping SHI, Wen-yu CUI, Yan-fang ZHANG, and Hai WANG designed the research; Jin-da WANG, Jing YIN, Zhi-yuan PAN, and Yong-ping SHI performed the research; Jing YIN and Yan-fang ZHANG contributed new reagents; Jin-da WANG, Wen-yu CUI, and Zhi-yuan PAN analyzed data; Jin-da WANG, Yong-ping SHI, and Hai WANG wrote the paper.

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