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The anti-diabetic effects and pharmacokinetic profiles of bis(maltolato)oxovanadium in non-diabetic and diabetic rats

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Abstract

The purpose of this study was to evaluate the anti-diabetic effects and pharmacokinetics of bis(maltolato)oxovanadium (BMOV) in rats. The anti-diabetic study was carried out in non-diabetic and diabetic rats by single-dose subcutaneous and intragastric administration. Pharmacokinetic investigation was performed using non-diabetic rats. Results showed that BMOV significantly decreased plasma glucose levels in diabetic rats at all given doses, and restored hyperglycaemic values to normal values after subcutaneous injections at doses of 4 and 8 mg vanadium (V)/kg or after intragastric administration at doses of 14 and 28 mgV/kg, respectively, but did not affect the plasma glucose level in non-diabetic rats. BMOV could be rapidly absorbed, slowly eliminated from plasma, widely distributed in various tissues and accumulated to a greater extent in the femur tissue. The average absolute bioavailability for intragastric administration at a single dose of 3, 6 and 12 mgV/kg was 28.1%, 33.7% and 21.4%, respectively. The presence of the peak vanadium level in the plasma was not coincident with that of the maximum effect of lowering plasma glucose levels. In conclusion, at the present dosing levels and administration routes, BMOV was effective in lowering plasma glucose levels in diabetic rats. BMOV has a promising outlook as an oral glucose-lowering drug.

Introduction

The number of patients with diabetes mellitus (DM) is predicted to increase globally to approximately 200 million within the next few years. DM is a chronic metabolic disease and classified as either type 1 or type 2. DM is threatening because of the development of many severe secondary complications, including atherosclerosis, microangiopathy, renal dysfunction and failure, cardiac abnormalities, diabetic retinopathy and ocular disorders (Sakurai 2002). So far, therapy for type 1 DM requires daily subcutaneous injections of insulin in conjunction with dietetics and ergotherapy, which cause pain and stress to the patients (Monaco et al 1996). Synthesized oral drugs are used to treat type 2 DM. There is an urgent need to investigate orally active therapeutic agents to replace painful insulin injections for type 1 DM and synthetic drugs with less or no side effects for type 2 DM.

Since the finding in 1977 that vanadate (+5) efficiently inhibits Na^+-K^+-ATPase, as well as other related phosphohydrolyses (Cantley et al 1977), investigations on vanadium have been focused on the biochemical and pharmacological roles of this metal ion. In particular, the finding in 1980 that the vanadate ion has an insulin-like effect generated considerable enthusiasm for its potential therapeutic application in human DM (Dubyak & Kleinzeller 1980). Since the last decade of the twentieth century, vanadium ions and complexes have been demonstrated to exert various insulin-mimetic and anti-diabetic effects, such as enhancing glucose transport and metabolism in adipocytes, hepatocytes and skeletal muscle, stimulating glycogen synthesis and lipogenesis, and inhibiting lipolysis and protein catabolism, in type 1 and 2 DM animal and human subjects (Shechter 1990; Cohen et al 1995; Goldfine et al 1995; Tsiani & Fantus 1997). However, the inorganic vanadium salts are considered less active and more toxic (Srivastava 2000). To reduce the toxicity and improve the absorption, tissue uptake and efficiency of vanadium, an organic

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ligand with less, or no, toxicity is of great interest. A lot of organic vanadium complexes have been synthesized and demonstrated to be effective (Srivastava & Mehdi 2005). Maltol, an approved food additive in Canada, the UK and the USA, is a desirable ligand because of its lack of toxicity and its ability to mobilize various metal ions in neutrally charged, water-soluble coordination complexes (Thompson et al 2006). By combining vanadium and maltol, bis(maltolato)oxovanadium (BMOV) has been synthesized and demonstrated to be effective and nontoxic over a six-month period of administration in diabetic rats and to be two to three times more potent than inorganic vanadium (Yuen et al 1993, 1995).

In this study, the glucose-lowering activity of BMOV in non-diabetic and diabetic rats through subcutaneous injection and intragastric administration routes was evaluated, and the corresponding vanadium concentrations in the plasma and tissues were measured. In addition, the pharmacokinetics of BMOV in rats was performed to analyse the pharmacokinetic behaviour of vanadium, establish a full model describing the fate of vanadium and determine absolute bioavailability.

Materials and Methods

Animals and chemicals

Male Sprague–Dawley rats, 220–260 g, were obtained from the Experimental Animal Center of Peking University, and maintained on a light–dark cycle. All rats were kept under conditions of 25°C and 50% relative humidity and were allowed free access to standard rat chow and water. Rats were acclimatized for 7 days before the induction of diabetes. All of the animal experiments were performed to the principles and guidelines of care and use of laboratory animals and approved by the Institutional Animal Care and Use Committee of Peking University.

BMOV was synthesized according to the published procedures (McNeill et al 1992). Alloxan was purchased from Sigma (St Louis, MO). The atomic absorption standard for vanadium (1 mg mL^{-1}) was available from the Central Iron & Steel Research Institute (Beijing, China). Nitric acid (guaranteed reagent) was a product of Beifang Jingxi Chemical Plant (Beijing, China) and perchloric acid (guaranteed reagent) was from Dongfang Chemical Plant (Tianjin, China). All other chemicals used were of analytical grade and were commercially available.

Anti-diabetes study

Induction of diabetes

To induce diabetes, Sprague–Dawley rats were fasted overnight for 12 h and given freshly prepared alloxan (150 mg kg^{-1}) in physiological saline by daily intraperitoneal injection for two consecutive days. Blood samples for monitoring plasma glucose were collected from tail veins at 48 h after the injection of alloxan solution. Rats showing plasma glucose levels >13 mM were considered to be diabetic and diabetic rats with consecutive seven-day hyperglycaemia (≥13 mM) were included for the experiment.

Subcutaneous injection

Fourteen non-diabetic rats were equally divided into two groups: Group I and Group II. The rats in Group I were given physiological saline as a saline control and the rats in Group II were given BMOV at a single dose of 4 mg vanadium (V)/kg as a treated non-diabetic control.

Twenty-eight diabetic rats were equally divided into four groups: Group III, Group IV, Group V and Group VI. Diabetic rats in Group III were administered physiological saline as a diabetic control, and diabetic rats in Group IV, V and VI were treated with BMOV at a single dose of 2, 4 and 8 mg V/kg, respectively.

Intragastric administration

BMOV was suspended in 3% gum Arabic. Fourteen non-diabetic rats were equally divided into two groups: Group VII and Group VIII. The rats in Group VII were given 3% gum arabic and the rats in Group VIII were given BMOV at a single dose of 14 mg V/kg.

Twenty-eight diabetic rats were equally divided into four groups: Group IX, Group X, Group XI and Group XII. The diabetic rats in Group IX were given 3% gum arabic and the diabetic rats in Group X, XI and XII were treated with BMOV at a single dose of 7, 14 and 28 mg V/kg, respectively.

Sampling and measurement

Blood samples obtained once daily during the seven-day treatment period were immediately placed in heparinized centrifuge tubes and centrifuged at 3000 *g* for 5 min. The plasma was harvested for analysis of plasma glucose and vanadium levels. Glucose levels were measured using biochemical kits purchased from Zhongsheng Biotech Inc. (Beijing, China).

All rats were sacrificed on the seventh day post-dosing. Vanadium levels in the plasma, hearts, livers, lungs, stomachs, brains, pancreata, spleens, kidneys, small intestines, tibialis anteriors and femurs were determined using the graphite furnace atomic absorption spectrometry (GFAAS) method according to our previous method (Zhang et al 2005).

Calibration curves were constructed over the range 20–200 ng mL^{-1}. The higher vanadium concentrations in samples (>200 ng mL^{-1}) were appropriately diluted to this range and then measured. Samples were prepared as follows: an aliquot of 100 μL of plasma or accurately weighed tissue specimen was digested in a blend of nitric acid and perchloric acid (3:1, v/v) and kept at room temperature for 12 h and then at 180°C for 48 h. The remaining acid solution was diluted to 1 mL with tri-distilled water and then directly assayed for measurement of the vanadium level. The detection limit was 0.7 ng mL^{-1} and the mean recovery was 101.2%. The coefficient of variation for the 20.0, 80.0 and 200.0 ng mL^{-1} vanadium solutions (3 replicates for each) was 6.7%, 3.4% and 2.7%, respectively.

Pharmacokinetics

Administration and sampling

Male healthy Sprague–Dawley rats were used for pharmacokinetic research, with five rats in each group, and fasted

overnight for 12 h before the experiment. BMOV dissolved in physiological saline was given to rats through the tail intravenously (3 mg V/kg) and subcutaneously (3 mg V/kg) and intragastrically (3 mg V/kg, 6 mg V/kg, and 12 mg V/kg). Blood samples were taken from tail veins at 0 (before dosing), 0.16, 0.5, 1, 2, 4, 6, 8, 10, 12, 24, 36, 48, 72, 96, 120, 144 and 168 h after drug administration. The plasma was separated by centrifugation at 3000 *g* for 5 min and stored at −20°C until the analysis of vanadium levels using the GFAAS method.

Calculation

Vanadium half-lives were calculated from concentration–time data using the 3P87 software, a practical pharmacokinetic program (the Chinese Society of Mathematical Pharmacology, Beijing, China). The area under the plasma vanadium curve (AUC) was calculated using the trapezoidal rule without extrapolation. The maximal vanadium concentration (C_{max}) and the time to reach C_{max} (T_{max}) were the actual observed values.

Statistics

Data were presented as mean ± standard deviation (s.d.). Analysis of variance was used to determine significance among groups, after which post-hoc tests with the Bonferroni correction were used for comparison between individual groups. $P < 0.05$ was considered to be significant.

Results

Anti-diabetic study

The mean plasma glucose level–time profiles after the subcutaneous injection of BMOV at a single dose and the intragastric administration of BMOV at a single dose are shown in Figures 1A and 2A, respectively. When the diabetic rats were treated with physiological saline, the plasma glucose levels fluctuated at the higher levels in the range 19–31 mM during the 7-day treatment period, indicating that the diabetic rat model induced by alloxan was stable (Group III, Figure 1A; Group IX, Figure 2A). The plasma glucose levels of non-diabetic rats treated with or without BMOV were maintained within normal glucose level values during the seven-day period, demonstrating that the plasma glucose levels of non-diabetic rats were not affected by BMOV (Group I and II, Figure 1A; Group VII and VIII, Figure 2A). Significant reduction in the plasma glucose level in the diabetic rat groups was observed after subcutaneous injection of BMOV at a single dose of 4 or 8 mg V/kg (Group V and VI, Figure 1A) and intragastric administration at a single dose of 14 or 28 mg V/kg (Group XI and XII, Figure 2A). The maximal hypoglycaemic effect occurred on the 2nd or 4th day for intragastric or subcutaneous administration, respectively. After the subcutaneous injection of BMOV 4 mg V/kg and the intragastric administration of BMOV 14 mg V/kg to non-diabetic and diabetic rats, the maximum vanadium concentrations were observed on the first day and then gradually decreased (Figure 1B, 2B). The presence of the peak

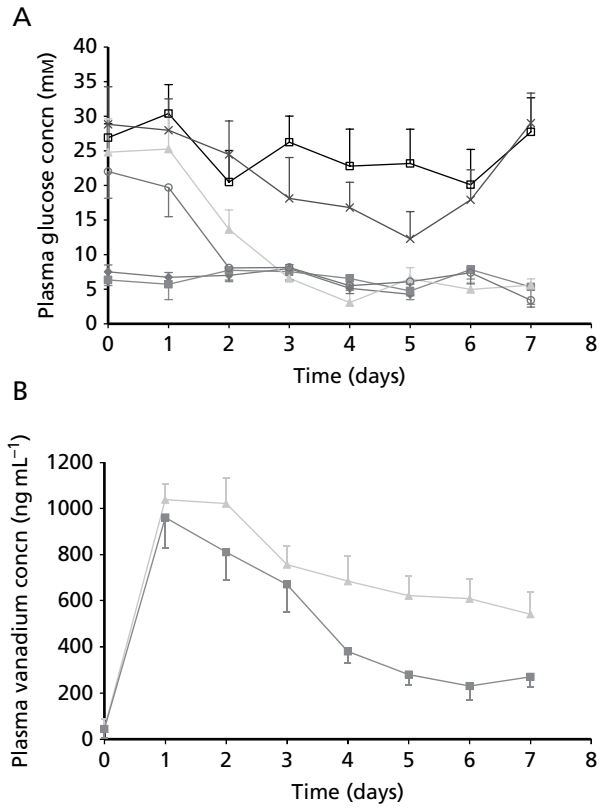

Figure 1 Mean plasma glucose level (A) and plasma vanadium concentration (B) in non-diabetic or diabetic rats following single subcutaneous injection of physiological saline (0.9% NaCl) or bis(maltolato)oxovanadium. Data are presented as the mean ± s.d., n = 7. Closed diamonds, Group I, non-diabetic, 0.9% NaCl; closed squares, Group II, non-diabetic, 4 mg V/kg; open squares, Group III, diabetic, 0.9% NaCl; crosses, Group IV, diabetic, 2 mg V/kg; closed triangles, Group V, diabetic, 4 mg V/kg; open circles, Group VI, diabetic, 8 mg V/kg.

vanadium levels in the plasma was not coincident with that of the maximum effect of lowering plasma glucose levels. On the seventh day post-dosing, the tissue vanadium levels in non-diabetic and diabetic rats treated with subcutaneous injections of BMOV 4 mg V/kg and intragastric administration of BMOV 14 mg V/kg are illustrated in Figure 3A and 3B. The highest vanadium concentrations were observed in the femurs, followed by the kidneys. The vanadium concentrations in the femurs were 3- to 5-fold higher than those in the kidneys. The vanadium levels in other tissues were relatively lower.

Pharmacokinetics

Plasma vanadium concentration–time profiles following intravenous, subcutaneous or intragastric administration are shown in Figure 4. After intravenous administration of 3 mg V/kg, vanadium levels in plasma declined from 2940 ± 186 ng mL^{-1} at 0.16 h to 1125 ± 118 ng mL^{-1} at 12 h, but decreased slowly from 1050 ± 28 ng mL^{-1} at 24 h to 444 ± 143 ng mL^{-1} at 168 h. After subcutaneous injection of 3 mg V/kg or

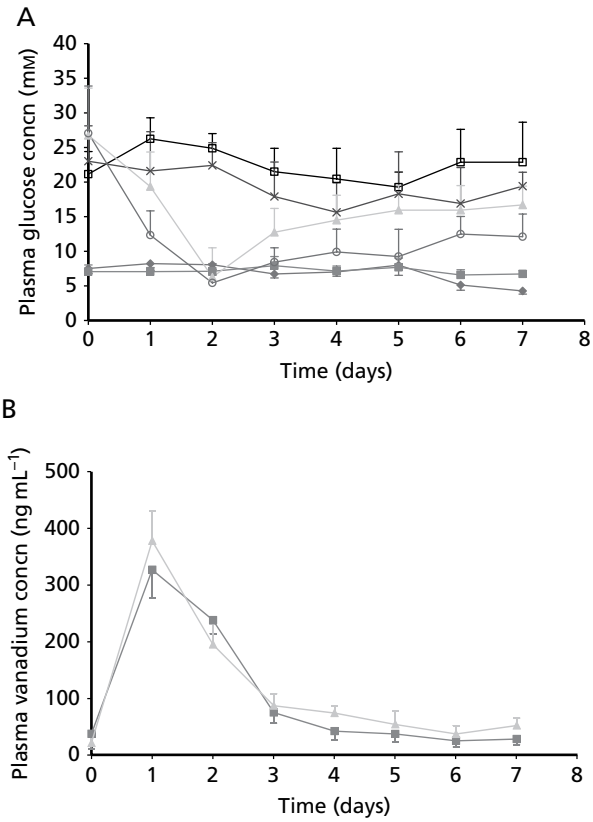

Figure 2 Mean plasma glucose level (A) and plasma vanadium concentration (B) in non-diabetic or diabetic rats following single intragastric injection of physiological saline (0.9% NaCl) or bis(maltolato)oxovanadium. Data are presented as the mean ± s.d., n = 7. Closed diamonds, Group VII, non-diabetic, 0.9% NaCl; closed squares, Group VIII, non-diabetic, 14 mg V/kg; open squares, Group IX, diabetic, 0.9% NaCl; crosses, Group X, diabetic, 7 mg V/kg; closed triangles, Group XI, diabetic, 14 mg V/kg; open circles, Group XII, diabetic, 28 mg V/kg.

intragastric administration of 3 mg V/kg, vanadium plasma levels increased to maximal values within 1–2 h, and declined to less than 1000 ng mL^{-1} at 12 h, then slowly decreased up to 168 h. Similar concentration–time profiles were observed after intragastric administration of 6 and 12 mg V/kg.

A one-compartment model was employed to calculate the half-life values due to the smaller Akaike information criterion (AIC) values compared with that of a two-compartment model. The pharmacokinetic parameters in rat plasma following administrations are shown in Table 1, and the results showed that the $AUC_{0\text{-}168\,h}$ of subcutaneous administration at a single dose of 3 mg V/kg was very significantly lower than that of intravenous administration at a single dose of 3 mg V/kg ($P < 0.01$), but markedly higher than that of intragastric administration at a single dose of 3 mg V/kg ($P < 0.01$). As compared with the intravenous administration at a single dose of 3 mg V/kg, the average absolute bioavailability for intragastric administration of BMOV at a single dose of 3, 6 or 12 mg V/kg was 28.1%, 33.7% and 21.4%, respectively, and the average absolute bioavailability for subcutaneous administration at a single dose of 3 mg V/kg was 45.6%. When compared with the same dosing groups (3 mg V/kg), the bioavailability of the subcutaneous injection was higher than that of the intragastric administration (45.6% for subcutaneous injection vs 28.1% for intragastric administration). The $AUC_{0\text{-}168\,h}$ values for the intragastric administration (3.58 ± 0.21, 8.57 ± 0.85 and $10.91 \pm 1.32 \times 10^4$ h ng mL^{-1}) linearly correlated ($r = 0.923$) with the doses (3, 6 and 12 mg V/kg). At a single dose of 3 mg V/kg, the average times to reach the maximal concentration were 0.6 ± 0.4 h for the subcutaneous administration and 2.4 ± 0.9 h for the intragastric administration, and average maximal concentrations were 1980 ± 150 ng mL^{-1} and 1420 ± 193 ng mL^{-1}, respectively. The maximal concentrations for intragastric administration elevated with the increased doses. The average elimination half-lives ($T_{1/2,ke}$) at a single dose of 3 mg V/kg were 109.2 ± 26.5 h for intravenous administration, 78.95 ± 14.9 h for subcutaneous administration and 91.9 ± 17.6 h for intragastric administration, respectively. The average elimination half-lives ($T_{1/2,ke}$) for intragastric administration at a single dose of 6 or 12 mg V/kg were very similar to that at a single dose of 3 mg V/kg.

Discussion

No diarrhoea or significant body weight decrease was observed in rats treated with BMOV at all given doses. This study showed that the organic vanadium compound BMOV effectively ameliorated the hyperglycaemia in diabetic rats. The hypoglycaemic effects of BMOV were not as potent as that of vanadyl acetylacetonate (VAC) (Zhang et al 2005), which was consistent with the results of Reul et al (1999). Yuen et al (1995, 1999) reported that the 50% effective dose (ED50) of BMOV at a single oral dose to Zucker diabetic fatty rats and streptozotocin-induced diabetic rats was 0.18 mmol kg^{-1} (9.2 mg V/kg) and 0.55 mmol kg^{-1} (28.1 mg V/kg), respectively. In our investigation, the plasma glucose in 100% of alloxan-induced diabetic rats was restored to the normal range on the third day after intragastric administration of BMOV at a single dose of 14 or 28 mg V/kg. After the intragastric administration of 28 mg V/kg or subcutaneous injection of 4 and 8 mg V/kg to diabetic rats, the euglycaemic effects lasted to the 5th or 7th day after administration, respectively, possibly because the accumulated vanadium in the bones, kidneys, livers or other tissues gradually released and exerted a longer action. Compared with other tissues, the bones had the highest vanadium concentration, which was consistent with the observation of Setyawati et al (1998). There was no distinct correlation between plasma vanadium concentrations and the reduction in plasma glucose levels in diabetic rats. Similar observations of BMOV were obtained by other investigators (Willsky et al 2001; Thompson et al 2003). These results suggest that the glucose-lowering mechanism of BMOV involves some sort of cascade effect; in other words, vanadium's interaction with endogenous biomolecules sets in motion a chain of events that continues independently of high concentrations of vanadium in the bloodstream.

Our results showed that T_{max} values of vanadium between BMOV and VAC after a subcutaneous injection at a single

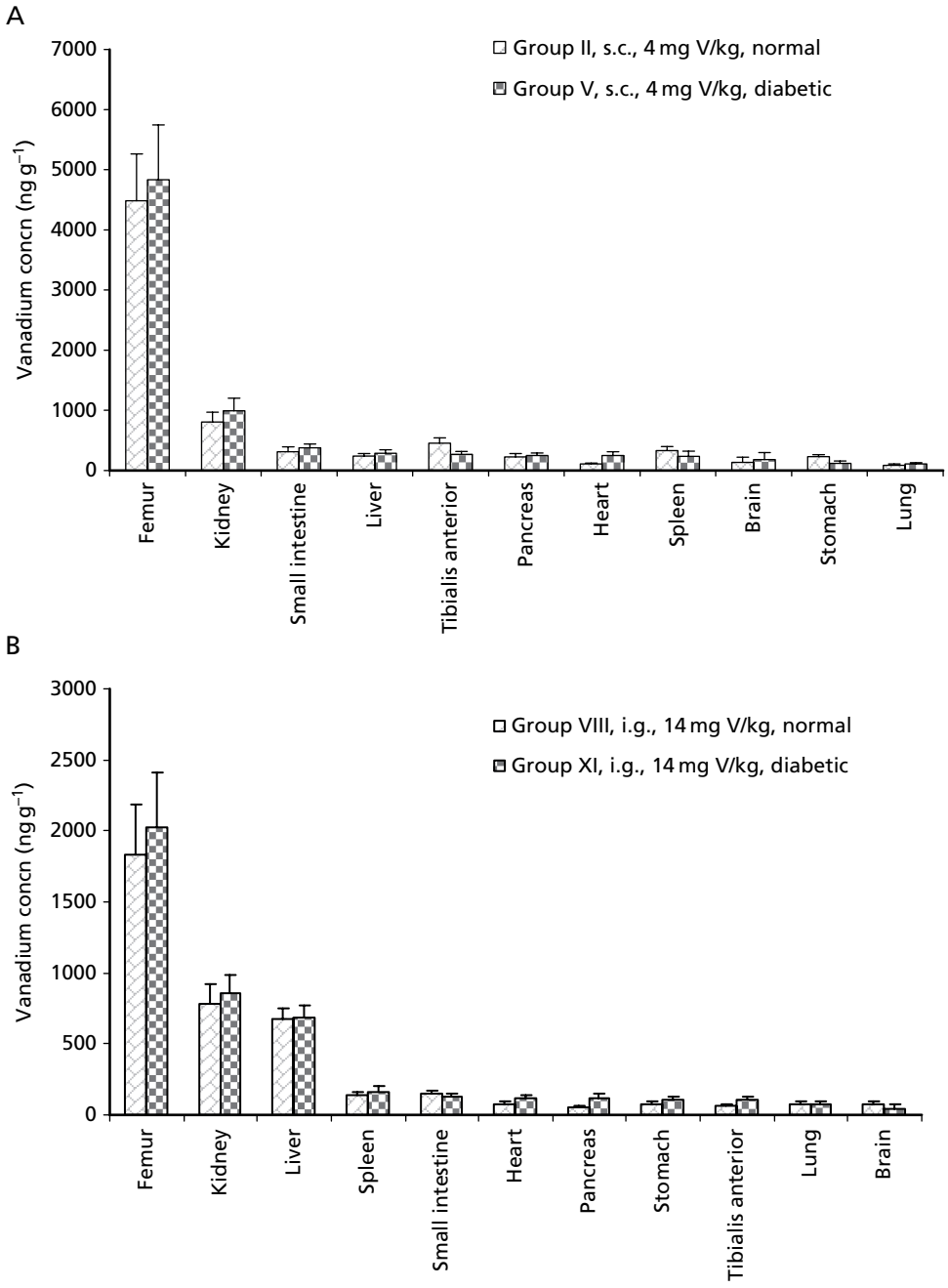

Figure 3 Vanadium concentrations in tissues on the seventh day after subcutaneous injection of bis(maltolato)oxovanadium at a single dose of 4 mg V/kg (A) or intragastric administration of bis(maltolato)oxovanadium at a single dose of 14 mg V/kg (B) to non-diabetic or diabetic rats. Data are presented as the mean ± s.d., n = 7.

dose of 3 mg V/kg and intragastric administration at a single dose of 3 or 6 mg V/kg were not significantly different (VAC: 0.9 ± 0.3 h for s.c. 3 mg V/kg, 3.0 ± 0.9 h for i.g. 3 mg V/kg, and 1.7 ± 0.3 h for i.g. 6 mg V/kg, refer to Zhang et al (2005)). T_{max} values of BMOV and VAC seemed to indicate that the different ligands (i.e. maltol and acetylacetone) similarly contributed to the absorption rates of BMOV and VAC. At a single oral dose of 3 or 6 mg V/kg, the absolute bioavailabilities of BMOV and VAC were close (VAC: 34.7% for 3 mg V/kg, 28.1% for 6 mg V/kg). The absolute bioavailabilities of vanadyl sulfate (VS), bis(picolinato)oxovanadium ($VO(pic)_2$) and bis(6-methylpicolinato)oxovanadium ($VO(6mpa)_2$) were 4.8%, 5.3% and 9.8%, respectively (Fugono et al 2001). These bioavailability values indicated that various ligands of vanadium compounds affected the affinity of vanadium to protein in plasma (Nagaoka et al 2002; Liboiron et al 2005).

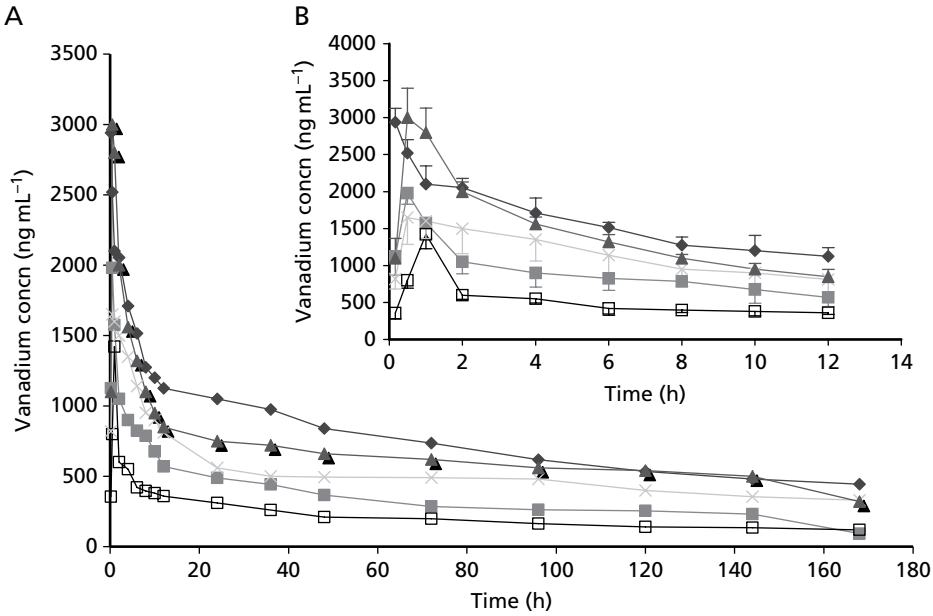

Figure 4 Mean plasma vanadium concentration–time profiles in non-diabetic rats following single intravenous injection of bis(maltolato)oxovanadium (equivalent to 3 mg V/kg), single subcutaneous injection of bis(maltolato)oxovanadium (equivalent to 3 mg V/kg) or single intragastric administration of bis(maltolato)oxovanadium (equivalent to 3, 6 or 12 mg V/kg). A. Whole plasma vanadium concentration–time profiles. B. Enlarged view for the profiles from 0 to 12 h. Data are presented as the mean ± s.d., n = 5. Closed diamonds, intravenous 3 mg V/kg; closed squares, subcutaneous 3 mg V/kg; open squares, intragastric 3 mg V/kg; crosses, intragastric 6 mg V/kg; closed triangles, intragastric 12 mg V/kg.

Table 1 Vanadium pharmacokinetic parameters in rat plasma after a single dose of bis(maltolato)oxovanadium through intravenous, subcutaneous or intragastric administration

	$AUC_{0\text{-}168\,h}$ ($\times 10^4$ h ng mL^{-1})	T_{max} (h)	C_{max} (ng mL^{-1})	$T_{1/2,\,ke}$ (h)
Intravenous 3 mg V/kg	12.73 ± 0.93	/	/	109.2 ± 26.5
Subcutaneous 3 mg V/kg	5.80 ± 0.49**	0.6 ± 0.4	2047 ± 411	78.95 ± 14.9
Intragastric 3 mg V/kg	3.58 ± 0.21$^{\#\#}$	2.4 ± 0.9$^{\#\#}$	1455 ± 267	91.9 ± 17.6
Intragastric 6 mg V/kg	8.57 ± 0.85	1.3 ± 0.7	1921 ± 358	93.1 ± 13.9
Intragastric 12 mg V/kg	10.91 ± 1.32	1.1 ± 0.5	3251 ± 543	90.6 ± 12.5

Data are presented as the mean ± s.d., n = 5. / represents none; $^{**}P < 0.01$, vs intravenous administration; $^{\#\#}P < 0.01$, vs subcutaneous administration.

Conclusion

BMOV was an effective plasma glucose-lowering agent in diabetic rats. It could be rapidly absorbed and T_{max} values ranged from 0.6 ± 0.4 h for subcutaneous injection to 2.4 ± 0.9 h for intragastric administration. The average absolute bioavailability for intragastric administration at a single dose of 3, 6 and 12 mg V/kg was 28.1%, 33.7% and 21.4%, respectively. BMOV widely distributed in various tissues and accumulated mainly in the femur tissue. There was no correlation between plasma vanadium concentration and plasma glucose lowering, and the accumulated vanadium in the bones, kidneys or other tissues may gradually release and exert a longer action. The potential toxicity issue that BMOV was slowly eliminated from the plasma and accumulated in the bones, kidneys and livers needs further investigation.

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