Pharmacokinetic study on gastrointestinal absorption of insulinomimetic vanadyl complexes in rats by ESR spectroscopy

Jun Fugono, Hiroyuki Yasui and Hiromu Sakurai

Abstract

Recently, we have shown that oral administrations of vanadyl ($+4$ oxidation state of vanadium) complexes normalize the blood glucose level of streptozotocin-induced diabetic rats (STZ-rats). To develop clinically useful insulin-mimetic vanadyl complexes, clarification of the pharmacokinetic features of vanadyl compounds is essential. First, we investigated the absorption processes of three compounds, an ionic form of vanadyl sulfate (VS) and the complex forms of bis(picolinato)oxovanadium(IV) ($VO(pic)_2$) and bis(6-methylpicolinato)oxovanadium(IV) ($VO(6mpa)_2$), from the gastrointestinal tract of healthy rats. The concentration curves of paramagnetic vanadyl species in the blood of rats after oral administration of these compounds, as monitored by X-band electron spin resonance (ESR) spectroscopy, exhibited biphasic increasing patterns, indicating that these compounds were absorbed from more than two sites in the gastrointestinal tract. The bioavailability of the compounds was enhanced in the following order on both oral and intraperitoneal administration: $VO(6mpa)_2 > VO(pic)_2 > VS$. In addition, bioavailability of the $VO(6mpa)_2$ on ileal administration was enhanced compared with that using other administration sites such as the stomach and jejunum, and resulted in an enhancement about 1.8 fold that compared with oral administration. On the basis of these results, we concluded that the bioavailability of the complex is enhanced most effectively by delivery of the $VO(6mpa)_2$ complex to the ileum.

Introduction

The number of patients with diabetes mellitus is predicted to increase globally to approximately 200 000 000 within the next few years. Abnormalities of glucose, protein and lipid metabolism in the organs, owing to chronic hyperglycaemia caused by the absolute or relative lack of insulin, develop into complications of diabetes mellitus.

Diabetes mellitus is classified as either type 1 (insulin-dependent diabetes mellitus; IDDM) and type 2 (non-insulin-dependent diabetes mellitus; NIDDM) (National Diabetes Data Group 1979). The former results from an absolute lack of insulin caused by a functional defect in the β cells of the Langerhans islands in the pancreas, and the latter from a relative lack of insulin secretion or the decline of insulin sensitivity in targeting organs (Kuzuya & Matsuda 1997). Therapy for NIDDM consists of dietetics and oral antihyperglycaemic agents, including sulfonylureas and biguanides. Sulfonylureas such as tolbutamide (Henquin 1980), chlorpropamide (Fine & Shedrovilzky 1970), acetohexamide (Jackson & Bresseler 1982) and glibenclamide (Meissner & Atwater 1976) stimulate insulin secretion

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(Sanz-Paris et al 1998). Biguanides such as metformin (Charles & Eschwege 1999) and buformin (Scheen & Lefebvre 1995) enhance insulin activity. IDDM patients require daily subcutaneous injections of insulin in conjunction with dietetics and ergotherapy (Monaco et al 1996). With the aim of relieving patients of the pain and stress of daily subcutaneous injections of insulin (Alberti 1977), the development of orally active insulin replacements or mimetics is under active investigation.

Since the finding in 1977 that vanadate ($+5$ oxidation state of vanadium) inhibits Na^+,K^+-ATPase (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 much interest (Dubyak & Kleinzeller 1980). We have attempted to develop new insulin-mimetic vanadyl ($+4$ oxidation state of vanadium) complexes that are more active and less toxic than vanadate (Sakurai et al 1990a, b, 1995, 1999; Watanabe et al 1994; Fujimoto et al 1997). The bis(6-methylpicolinato)oxovanadium(IV) (VO(6mpa)$_2$) complex has been proposed to be the most effective on oral administration to treat diabetic animals with streptozotocin-induced IDDM (STZ-rats) (Fujimoto et al 1997) and also with hereditary NIDDM (KKA[y] mice) (Fujisawa & Sakurai 1999). Other researchers have proposed a different type of vanadyl complex with maltol as a food additive (McNeill et al 1992; Yuen et al 1993, 1995; Reul et al 1999).

Metal ions and metal complexes generally have a toxic effect on physiological functions when given in an overdose. For example, gastrointestinal side effects including diarrhoea and vomiting were reported when an overdose of vanadium was given to humans (Dafnis & Sabatini 1994). Therefore, the development of complexes that are more active, less toxic and with fewer side effects is an important consideration in the treatment of diabetes mellitus.

Because the VO(6mpa)$_2$ complex has been found to be effective in treating both types of diabetes, understanding its absorption characteristics in the digestive organs and determining the most suitable dosage form are essential steps toward its future clinical use. In this study, we investigated the gastrointestinal absorption characteristics of the paramagnetic vanadyl species in healthy rats which were given an ionic form of vanadyl sulfate (VS) and the complex forms of bis-(picolinato)oxovanadium (VO(pic)$_2$) and bis(6-methylpicolinato)oxovanadium (VO(6mpa)$_2$) (Figure 1) after oral, intrajejunal, and intra-ileal administrations by pharmacokinetic analysis using an X-band electron spin resonance (ESR) method. Vanadyl species have been
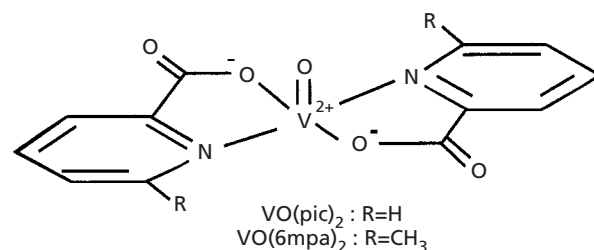
Figure 1 Structures of vanadyl compounds: bis(picolinato)oxovanadium(IV) (VO(pic)$_2$) and bis(6-methylpicolinato)oxovanadium(IV) (VO(6mpa)$_2$).

found to be stable in biological systems such as several tissues and blood and the main chemical form in rats (Sakurai et al 1980, Yasui et al 2000), and also exhibit antidiabetic activity in experimental animals (Sakurai et al 1990a; 1990b, Nakai et al 1995, Fujimoto et al 1997, Fujisawa & Sakurai 1999). We therefore successfully used X-band ESR spectrometry to determine the concentrations of vanadyl species in the blood of rats after administration of three vanadyl compounds.

Materials and Methods

Materials

Vanadyl sulfate (VOSO$_4$·6.2H$_2$O) (VS) was purchased from Nacalai Tesque Co. (Kyoto, Japan), and its purity was determined by chelatometry using EDTA-Na$_2$ and Cu-PAN (Cu-1-(2-pyridylazo)-2-naphthol complex) (Dojindo, Kumamoto, Japan). Picolinic acid and acacia were purchased from Wako Pure Chemicals (Osaka, Japan) and 6-methylpicolinic acid from Tokyo Kasei Organic Chemicals (Tokyo, Japan). Pentobarbital (50 mg mL^{-1}) was purchased from Dainabot Co. (Osaka, Japan), sodium heparin from Simizu Experimental Material Co. (Kyoto, Japan) and Aron Alpha, an instant adhesive for surgery, from Sankyo Co. (Tokyo, Japan). Rat serum albumin (RSA) was purchased from Sigma Chemical Co. (St Louis, MO). All other materials used were of analytical reagent grade. Bis(picolinato)oxovanadium(IV) (VO(pic)$_2$) and bis(6-methylpicolinato) oxovanadium(IV) (VO(6mpa)$_2$) were synthesized in our laboratory (Sakurai et al 1995, Fujimoto et al 1997).

Animals

Male Wistar rats (8 weeks old, 240–250 g) were purchased from Simizu Experimental Material Co. (Kyoto, Japan). All experiments were approved by the Exper-

imental Animal Research Committee of Kyoto Pharmaceutical University and were performed according to the Guideline for Animal Experimentation of Kyoto Pharmaceutical University. Rats were fasted overnight for 12 h before experiments. VS and VO(pic)$_2$ were dissolved in physiological saline (0.9% NaCl) for administration. VO(6mpa)$_2$ was dissolved in 4% RSA solution for intravenous administration, and suspended in 5% acacia solution for other administrations.

Administration of vanadyl complexes to healthy rats

Intravenous administration
Each vanadyl compound was intravenously injected through the right femoral vein of the rat at a dose of 2 mg (39 μmol) of vanadium/kg body weight. Blood samples were periodically collected from the jugular vein by a puncture with a heparinized syringe with a 26 G needle, under light ether anaesthesia.

Intraperitoneal administration
Each solution of the three compounds was intraperitoneally administered to rats at a dose of 10 mg (196 μmol) of vanadium/kg body weight. Blood samples were collected by the same method as that used for the intravenous administration.

Oral administration
Each solution of the three compounds was intragastrically administered to rats using a stainless sonde at a dose of 10 mg (196 μmol) of vanadium/kg body weight. Blood samples were collected by the same method as that used for the intravenous administration.

Intrajejunal administration
For intrajejunal administration of the three compounds to the rats, an incision (2 cm) was made in the epigastrium and each solution was administered into the jejunum through the stomach under anaesthesia with pentobarbital (50 mg kg^{-1}) using a syringe with a 27 G needle. Each solution was injected into the jejunum at a dose of 1 mL kg^{-1}, corresponding to 10 mg (196 μmol) vanadium/kg body weight. The top of a needle was made smooth and positioned 3 cm distal to the point where the duodenum curves towards the midline. After the administration, the puncture made in the stomach wall was closed with a drop of tissue cement (Aron Alpha) and the abdominal incision was sutured. The rats awoke approximately 1 h after administration.

Blood samples were collected by the same method as that used for the intravenous administration.

Intra-ileal administration
For intra-ileal administration of the three compounds, an incision (2 cm) was made in the underbelly and each solution was administered into the ileum under anaesthesia with pentobarbital (50 mg kg^{-1}) using a syringe with a 27 G needle. The volume of each solution and the dose of vanadium were the same as those used for intrajejunal administration. The top of a needle was placed 25 cm proximal to the ileo-caecal junction. After the administration, the puncture made in the ileum was closed with a drop of tissue cement (Aron Alpha) and the abdominal incision was sutured. The rats awoke approximately 1 h after administration. Blood samples were collected by the same method as that used for the intravenous administration.

X-band ESR determination of vanadyl species in the blood of rats treated with vanadyl compounds

Electron spin resonance (ESR) spectroscopy is useful for quantitative determination of vanadyl species. ESR spectra of vanadyl species were measured with a JES-RE1X spectrometer (JEOL Tokyo, Japan) at room temperature under the following conditions: field modulation frequency, 100 kHz; modulation amplitude width, 2.0 mT; microwave power, 5.0 mW; magnetic field, 335 $\pm$ 50 mT; sweep time, 1 min; response, 0.03 s. The quartz capillary (Eiko-sha, Osaka, Japan) was used for measurement of blood samples. Storage and analysis of the ESR data were performed with an ESPRIT ESR Data System (JEOL, Tokyo, Japan).

Blood samples were measured on the same day as the blood sampling. Blood samples were reserved at 4°C until ESR measurement. A 20-μL portion of each blood sample was transferred to the quartz capillary, which was then fixed in the ESR cavity. To determine the concentrations of vanadyl species in the blood after each administration route, 20 μL of the blood of untreated rats containing each vanadyl compound was transferred to the quartz capillary for ESR measurement. By monitoring the signal intensities at the central peak corresponding to vanadyl compounds involving VS, VO(pic)$_2$, and VO(6mpa)$_2$ in fresh blood, three calibration curves were obtained. Linear relationships between ESR signal intensities and the four different concentrations of the three vanadyl compounds in blood

were found within the range 25–200 nmol mL^{-1}, where the correlation coefficients for the three vanadyl compounds with linear regression were greater than 0.998 for the four concentrations, in three repeated measurements. The detection limit at an S/N ratio of 3 was 10 nmol mL^{-1} for the three compounds. Several blood samples taken after intravenous and intraperitoneal administration, where concentrations were higher than 200 nmol mL^{-1}, were appropriately diluted with the blood of untreated rats to allow monitoring of those signal intensities in the 25–200 nmol mL^{-1} range.

Pharmacokinetic analysis of vanadyl species in the blood of rats

The time course of vanadyl concentration in the blood was evaluated on the basis of non-compartment pharmacokinetic analysis (moment analysis) (Yamaoka et al 1978). The area under the vanadyl concentration in the blood–time curve (AUC), maximal vanadyl concentration (C_{max}), mean residence time (MRT) of vanadyl species and time to reach C_{max} (T_{max}) were estimated model-independently. AUC and MRT after intravenous administration were calculated by trapezoidal integration with extrapolation. The values of total clearance (CL) and volume of distribution at steady state (Vd) of the three vanadyl compounds were estimated from the curves for the vanadyl concentrations in the blood after intravenous administration. The AUC and MRT after other routes of administration were calculated by the trapezoidal integration without extrapolation, because the time profiles around the terminal phase were too unstable to extrapolate. Bioavailability (Fa) in each group was calculated by the following equation:

$$Fa = AUC \cdot CL/Dose \qquad (1)$$

Statistical analysis

All experimental results were expressed as the arithmetic mean and standard deviation of measurements from three or four rats. Statistical analysis was performed using analysis of variance at a 5% ($P < 0.05$) or 1% ($P < 0.01$) significance level.

Results and Discussion

Because vanadyl ($+4$ oxidation state of vanadium) and vanadate ($+5$ oxidation state of vanadium) ions have been shown to have insulin-mimetic activity (Heyliger et al 1985; Sakurai et al 1990a), we focussed on the

vanadyl compounds that are less toxic and have higher bioavailability than vanadate. Since 1990, we have developed a variety of orally active vanadyl complexes to treat both types of diabetes mellitus (Sakurai et al 1995; Fujimoto et al 1997; Fujisawa & Sakurai 1999). In the USA in recent years, vanadium ions (vanadyl and vanadate) have been used clinically to treat diabetic patients by oral administration (Cohen et al 1995; Goldfine et al 1995a, b, 2000; Boden et al 1996; Halberstam et al 1996). However, such ionic forms of vanadium are less effective at treating diabetes in animals, probably owing to their poor gastrointestinal absorption. The oral absorption ratio of vanadium ions in humans was reported to be 0.1–0.5% as estimated by atomic absorption analysis (Nielsen 1995). Another report showed that the absorption ratio of soluble vanadium salt after oral administration in rats was approximately 10% by radioisotope experiments (Setyawati et al 1998). Subsequently, vanadyl complexes with improved absorption were prepared and found, on the basis of animal experiments, to be more useful than vanadyl ions in the treatment of IDDM (Sakurai et al 1995; Fujimoto et al 1997). Thus, it is necessary to understand the pharmacokinetic features of the vanadyl state in the blood of rats given vanadyl compounds, to establish parameters for their safe clinical use as antidiabetic agents in humans.

Of the various vanadyl complexes prepared, we selected VO(6mpa)$_2$ (Figure 1) (Fujimoto et al 1997), which was found to normalize serum glucose levels in streptozotocin-induced IDDM rats (STZ-rats) and in hereditary-NIDDM model (KKA^y) mice by intraperitoneal and oral administration (Fujisawa & Sakurai 1999). To compare the results from these groups and the control, an ionic form of VS and a complex form of VO(pic)$_2$ were also used.

Previously, it was reported that even if vanadyl is oxidized to vanadate by molecular dioxygen in the blood or serum, vanadate is rapidly re-reduced to vanadyl by endogenous reducing agents such as ascorbate and glutathione (Macara et al 1980; Ding et al 1994). In addition, it was shown that vanadyl ions specifically bind to albumin and transferrin as detected by ESR (Chasteen et al 1986). From these observations, we confirmed that vanadyl species are stable in the fresh blood and sera of rats, as well as in the protein-binding forms in the blood (Yasui et al 2000). It was also indicated that the disappearance of vanadyl ESR signals from the circulating blood is due to their transfer and distribution to the tissues and elimination from the body, and not due to the redox or protein-binding processes in the circulating blood (Yasui et al 2000). We
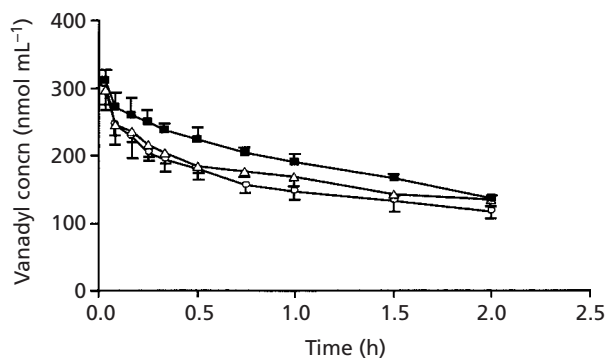
Figure 2 Time course for vanadyl concentration in the blood of rats after intravenous administration. Rats were given VS ($\bigcirc$), VO(pic)$_2$ ($\triangle$) or VO(6mpa)$_2$ ($\blacksquare$) at a dose of 39 μmol (2 mg) vanadium/kg body weight. Each symbol represents the mean $\pm$ s.d., n = 3 rats.
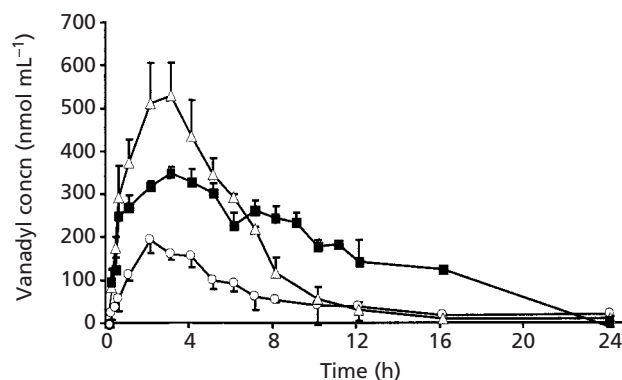
Figure 3 Time course for vanadyl concentration in the blood of rats after intraperitoneal administration. Rats were given VS ($\bigcirc$), VO(pic)$_2$ ($\triangle$) or VO(6mpa)$_2$ ($\blacksquare$) at a dose of 196 μmol (10 mg) vanadium/kg body weight. Each symbol represents the mean $\pm$ s.d., n = 3 rats.

also confirmed that the vanadyl species is the active form to exhibit antidiabetic activity in experimental animals (Sakurai et al 1990a, b; Nakai et al 1995; Fujimoto et al 1997; Fujisawa & Sakurai 1999). On the basis of these results, we used ESR in the present pharmacokinetic analysis of three vanadyl compounds.

VS, VO(pic)$_2$, and VO(6mpa)$_2$ were given by intravenous injection to rats at a dose of 39 μmol vanadium/kg body weight. The time course of vanadyl concentrations in the blood are shown in Figure 2, and the pharmacokinetic parameters obtained from the concentration curves are summarized as follows: AUC values, 697 ± 89 nmol h mL^{-1} for VS, 845 ± 59 nmol h mL^{-1} for VO(pic)$_2$ and 792 ± 16 nmol h mL^{-1} for VO(6mpa)$_2$; MRT values, 3.28 ± 0.76 h for VS, 3.80 ± 0.43 h for VO(pic)$_2$ and 2.90 ± 0.12 h for VO(6mpa)$_2$; CL values, 56.9 ± 7.7 mL h^{-1} kg^{-1} for VS, 46.6 ± 3.3 mL h^{-1} kg^{-1} for VO(pic)$_2$ and 49.5 ± 1.0 mL h^{-1} kg^{-1} for VO(6mpa)$_2$; and Vd values, 183 ± 22 mL kg^{-1} for VS, 176 ± 8 mL kg^{-1} for VO(pic)$_2$ and 144 ± 3 mL kg^{-1} for VO(6mpa)$_2$. The pharmacokinetic parameters of the three vanadyl compounds were not significantly dif-

ferent after intravenous administration. When the three compounds were given to rats by the intraperitoneal, oral, intrajejunal or intra-ileal routes, the administered dose was fixed at 196 μmol vanadium/kg body weight. CL values obtained from intravenous data were used for calculating the Fa value, which is defined as an absorption ratio from non-intravenous routes.

To evaluate the transfer of vanadyl compounds from the abdominal cavity into systemic circulation, each compound was given to rats by intraperitoneal injection. The time course of vanadyl concentration in the blood and the pharmacokinetic parameters obtained are shown in Figure 3 and Table 1, respectively. T_{max} values were almost the same for the three compounds, whereas a higher vanadyl concentration in the blood of rats treated with VO(6mpa)$_2$ was observed for an extended period (3–16 h) compared with the elevated periods of VS and VO(pic)$_2$ (Figure 3), indicating that the behaviour of VO(6mpa)$_2$ in the absorption process from the abdominal cavity to the systemic circulation is remarkably different from that of VS or VO(pic)$_2$. Fa values of rats treated with VS, VO(pic)$_2$ and

Table 1 Pharmacokinetic parameters in the absorption processes of VS, VO(pic)$_2$ and VO(6mpa)$_2$ after intraperitoneal administration

Compound	AUC (nmol h mL^{-1})	C$_{max}$ (nmol mL^{-1})	MRT (h)	T$_{max}$ (h)	Fa (%)
VS	1573 ± 109	203 ± 29	7.89 ± 0.22	2.00 ± 0.00	45.6
VO(pic)$_2$	3484 ± 284*	567 ± 47**	5.25 ± 0.17*	2.33 ± 0.58	82.8
VO(6mpa)$_2$	4248 ± 300*†	365 ± 34**†	7.99 ± 0.17††	3.00 ± 1.00	107.2

Data are shown as the mean values $\pm$ s.d. for 3 rats. *$P < 0.05$, **$P < 0.01$ vs VS; †$P < 0.05$, ††$P < 0.01$ vs VO(pic)$_2$.
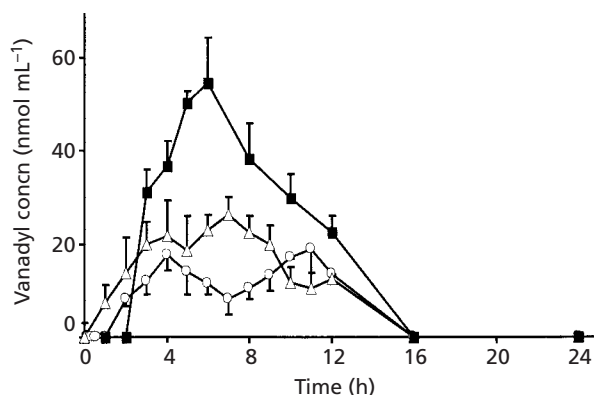
Figure 4 Time course for vanadyl concentration in the blood of rats after oral administration. Rats were given VS ($\bigcirc$), VO(pic)$_2$ ($\triangle$) or VO(6mpa)$_2$ ($\blacksquare$) at a dose of 196 μmol (10 mg) vanadium/kg body weight. Each symbol represents the mean $\pm$ s.d., n = 3 or 4 rats.

VO(6mpa)$_2$ were 45.6, 82.8 and 107.2%, respectively. C_{max} values increased in the following order: VO(pic)$_2$ (567 $\pm$ 47 nmol mL^{-1}) > VO(6mpa)$_2$ (365 $\pm$ 34 nmol mL^{-1}) > VS (203 $\pm$ 29 nmol mL^{-1}). MRT values of VS and VO(6mpa)$_2$were almost the same (7.89 $\pm$ 0.22 h and 7.99 $\pm$ 0.17 h, respectively), whereas that of VO(pic)$_2$ was the shortest (5.25 $\pm$ 0.17 h) (Table 1). Previously, we reported that a low dose of VO(6mpa)$_2$ normalizes the glucose levels of STZ-rats after intraperitoneal administration (Fujimoto et al 1997), suggesting that vanadyl species, after intraperitoneal administration of VO-(6mpa)$_2$, distribute and accumulate in the body and the blood more efficiently than other compounds. Higher blood concentrations of vanadyl species for an extended

period after VO(6mpa)$_2$ administration showed that the VO(6mpa)$_2$ can treat IDDM in STZ-rats at a lower dose than can VS or VO(pic)$_2$ (Fujimoto et al 1997).

To investigate the intestinal absorption of vanadyl compounds, we examined the vanadyl concentration profiles of the vanadyl compounds after oral administration (Figure 4 and Table 2). Fa values of rats given VS, VO(pic)$_2$ and VO(6mpa)$_2$ were estimated to be 4.8, 5.3 and 9.8%, respectively. The C_{max} of VO(6mpa)$_2$ was significantly higher (54.6 $\pm$ 9.7 nmol mL^{-1}) than that of VO(pic)$_2$ (26.2 $\pm$ 3.4 nmol mL^{-1}) or VS (18.9 $\pm$ 5.2 nmol mL^{-1}) (Table 2). The MRT values of the three vanadyl compounds were almost the same, whereas the T_{max} of VO(6mpa)$_2$ was the earliest (5.50 $\pm$ 0.58 h), followed by VO(pic)$_2$ (7.00 $\pm$ 1.00 h) and VS (8.33 $\pm$ 3.79 h). Interestingly, two absorption maxima were observed in the vanadyl concentration curves of VS and VO(pic)$_2$, while a single absorption maximum was found in that of VO(6mpa)$_2$ (Figure 4). The T_{max} values for the first maximum of VS and VO(pic)$_2$ were almost same (4 h), though the T_{max} for the second maximum of VO(pic)$_2$ was significantly earlier than that of VS. These results suggested that multiple absorption sites for these compounds are present in the gastrointestinal tract. When VO(6mpa)$_2$ suspended in saline containing 5% acacia was given orally to rats, the complex was shown to resolve in the intestinal lumen before absorption to the systemic circulation. Absorption from the upper intestine corresponding to the first absorption maximum was suppressed because of the low solubility of VO(6mpa)$_2$, and therefore a single absorption maximum corresponding to the lower intestine was observed. These results, on absorption of the vanadyl compounds in

Table 2 Pharmacookinetic parameters in the absorption processes of VS, VO(pic)$_2$ and VO(6mpa)$_2$ after oral, intrajejunal and intra-ileal administration

Compound	Administration site	AUC (nmol h mL^{-1})	C_{max} (nmol mL^{-1})	MRT (h)	T_{max} (h)	Fa (%)	Enhancement of Fa
VS	Stomach	165 $\pm$ 6	18.9 $\pm$ 5.2	7.93 $\pm$ 0.12	8.33 $\pm$ 3.79	4.8	1
	Jejunum	348 $\pm$ 5	47.8 $\pm$ 7.4	5.20 $\pm$ 0.03	2.67 $\pm$ 1.53	10.1	2.11
	Ileum	433 $\pm$ 57	31.5 $\pm$ 12.9	9.09 $\pm$ 0.41	1.50 $\pm$ 0.87	12.6	2.62
VO(pic)$_2$	Stomach	223 $\pm$ 12	26.2 $\pm$ 3.4*	6.87 $\pm$ 0.09*	7.00 $\pm$ 1.00	5.3	1
	Jejunum	442 $\pm$ 23	45.2 $\pm$ 1.2	8.13 $\pm$ 0.28*	4.67 $\pm$ 0.58	10.5	1.98
	Ileum	454 $\pm$ 43	56.6 $\pm$ 8.3*	7.44 $\pm$ 0.57*	4.00 $\pm$ 1.73	10.8	2.04
VO(6mpa)$_2$	Stomach	388 $\pm$ 52*†	54.6 $\pm$ 9.7*†	7.60 $\pm$ 0.20†	5.50 $\pm$ 0.58	9.8	1
	Jejunum	311 $\pm$ 65†	53.0 $\pm$ 3.6	7.30 $\pm$ 1.00*	6.50 $\pm$ 0.58*†	7.8	0.80
	Ileum	699 $\pm$ 9**†	99.3 $\pm$ 4.9**††	6.82 $\pm$ 0.11**	5.00 $\pm$ 0.00**	17.6	1.80

Data are shown as the mean values $\pm$ s.d. for 3 or 4 rats. *$P < 0.05$, **$P < 0.01$ vs VS at the same administration site; †$P < 0.05$, ††$P < 0.01$ vs VO(pic)$_2$ at the same administration site. Enhancement of Fa is calculated as (Fa value of rats given each compound at each administration site)/(Fa value after oral administration).
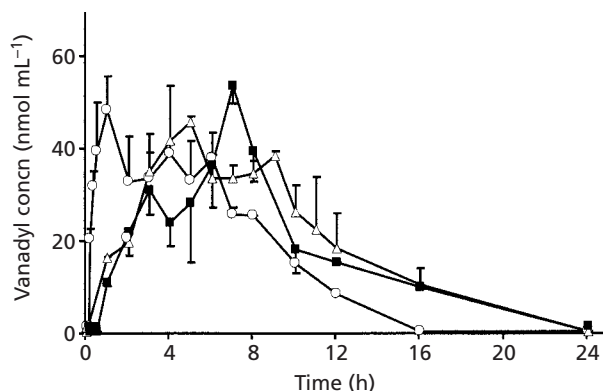
Figure 5   Time course for vanadyl concentration in the blood of rats after intrajejunal administration. Rats were given VS (○), VO(pic)$_2$ (△) or VO(6mpa)$_2$ (■) at a dose of 196 μmol (10 mg) vanadium/kg body weight. Each symbol represents the mean±s.d., n = 3 or 4 rats.
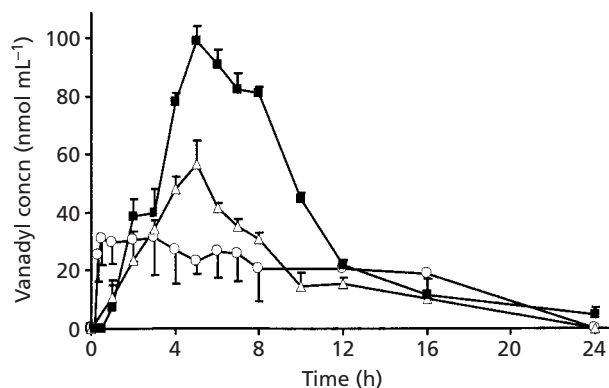
Figure 6   Time course for vanadyl concentration in the blood of rats after intra-ileal administration. Rats were given VS (○), VO(pic)$_2$ (△) or VO(6mpa)$_2$ (■) at a dose of 196 μmol (10 mg) vanadium/kg body weight. Each symbol represents the mean±s.d., n = 3 or 4 rats.

terms of Fa values, agree closely with those from a previous study in rats (Setyawati et al 1998).

Next, we examined the absorption of the vanadyl compounds after intrajejunal administration (Figure 5 and Table 2). The Fa and C_{max} values of rats treated with the three compounds were almost the same (Fa value, VS 10.1 %, VO(pic)$_2$ 10.5 % and VO(6mpa)$_2$ 7.8 %; C_{max} value, VS 47.8±7.4 nmol mL^{-1}, VO(pic)$_2$ 45.2± 1.2 nmol mL^{-1} and VO(6mpa)$_2$ 53.0±3.6 nmol mL^{-1}). The MRT of VS was significantly shorter (5.20±0.03 h) than that of VO(6mpa)$_2$ (7.30±1.00 h) or VO(pic)$_2$ (8.13±0.28 h). Similarly, the first T_{max} (2.67±1.53 h) of VS was significantly shorter than that of VO(pic)$_2$

(4.67±0.58 h) or VO(6mpa)$_2$ (6.50±0.58 h). Two absorption maxima were observed in each vanadyl concentration curve of the three compounds, and the second T_{max} of VS (5 h) was earlier than those of VO(pic)$_2$ and VO(6mpa)$_2$ (7 h).

Finally, the absorption of vanadyl compounds was examined after intra-ileal administration (Figure 6 and Table 2). The Fa value of rats given VO(6mpa)$_2$ intra-ileally was larger (17.6 %) than that of rats given the other two compounds. In addition, the C_{max} of VO-(6mpa)$_2$ was significantly larger (99.3±4.9 nmol mL^{-1}) than those of the other compounds. The MRT of VS after intra-ileal administration was longer (9.09± 0.41 h) than that of VO(pic)$_2$ (7.44±0.57 h) or VO-(6mpa)$_2$ (6.82±0.11 h) (Table 2), whereas the T_{max} of VS occurred earlier (1.50±0.87 hr) than for the other two complexes. The C_{max} and Fa values of the three compounds after intra-ileal administration were approximately twice those obtained after oral administration. When the three compounds were administered directly into the large intestine, the concentration of the vanadyl species in the blood was below the detection limit. Thus, because of the lack of absorption of vanadyl compounds from the large intestine, it is suggested that the single absorption maximum observed after intra-ileal administration arose from absorption of the compounds from the ileum.

After oral administration, the vanadyl complexes are supposed to be mostly decomposed in the stomach where the pH is 1.5–3.5 (Kararli 1995). Thus, vanadyl complexes given by direct intestinal administration (e.g. into the ileum) will be absorbed more thoroughly than those given orally, and will result in higher pharmacological activity.

In conclusion, we propose that dosage delivered at the ileum as an intact form (such as a capsule coated by pH-dependent disintegratable polymer) will improve the bioavailability of vanadyl complexes, and in turn, will increase the insulin-mimetic activity of the vanadyl complexes on oral administration. Research is underway to examine the bioavailability and the metabolism of the complexes in experimental animals with both types of diabetes mellitus.

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