Pharmacokinetic Study and Trial for Preparation of Enteric-Coated Capsule Containing Insulinomimetic Vanadyl Compounds: Implications for Clinical Use

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Abstract: To treat patients suffering from diabetes mellitus, we developed several types of orally active vanadyl complexes to replace painful insulin injections, and prepared them in the form of enteric-coated capsules containing vanadium compounds. Pharmacokinetic analysis demonstrated that these capsules enhance the bioavailability of pharmacologically active vanadyl species.

Keywords: Vanadyl compound, insulinomimetic activity, diabetes mellitus, blood circulation monitoring-electron paramagnetic resonance (BCM-EPR), pharmacokinetic analysis, bioavailability, enteric-coated capsule.

INTRODUCTION

Diabetes mellitus (DM) is predicted to be one of the most important diseases in the 21st century, due to rapid changes in lifestyles. DM is dreaded because of its severe complications, including atherosclerosis, microangiopathy, renal dysfunction and failure, cardiac abnormality, diabetes retinopathy and ocular disorders. DM is divided mainly into two classes: type 1 (insulin-dependent DM; IDDM) and type 2 (non-insulin-dependent DM; NIDDM) [1]. The former results from the complete absence of insulin synthesis and secretion caused by functional defects in B cells of the Langerhans islands in the pancreas. The latter results from a relative lack of insulin secretion or from decreased insulin sensitivity in the target organs [2]. Patients suffering from type 1 DM require daily subcutaneous injections of insulin, and the glucose level measurement before each injection. This kind of treatment causes physical and mental pain. Therapy for type 2 DM consists of dietetics and administration of oral antihyperglycemic agents such as sulfonylureas and biguanides, although sometimes type 2 DM also requires insulin injections.

Ever since the findings that vanadium compounds such as sodium vanadate ($NaVO_3$: +5 oxidation state of vanadium) [3, 4] and vanadyl sulfate ($VOSO_4$: +4 oxidation state of vanadium) [5, 6] were successfully used to treat hyperglycemia in experimental animals, researchers have had a keen interest in the relationship between DM and vanadium, as well as in the mechanisms underlying vanadium's action. As a result, new therapies using vanadium compounds have emerged [7-9].

The coordination of ligands with metal ions often enhances pharmacological activity of bioactive drugs or promotes new drug actions. A typical example is *cis*-diamine dichloro platinum (II) (cis-DDP or cisplatin) coordination compound, which has shown beneficial effects in the treatment of several types of cancer [10].

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Since 1995, simple vanadium compounds such as $VOSO_4$ and $NaVO_3$ have been clinically tested in humans bearing DM [11-15]. When orally administered at a dose of 150 mg (3 mmol) / day for 6 weeks, $VOSO_4$ has shown improvement in these subjects' type 2 DM in terms of plasma glucose, hemoglobin A_{1c} (HbA_{1c}), and fructosamine level [15]. Interestingly, before $VOSO_4$ treatment, subjects showed plasma vanadium levels below 10 μg / L (0.2 μmol / L) that increased to 104 ± 18 μg / L (2.0 ± 0.4 μmol / L) after the 6-week treatment. These results clearly indicated that the enhancement of plasma vanadium levels correlates with the improvement of the subjects' diabetic state. Such significant antidiabetic activity of orally administered $VOSO_4$ led us to investigate the administration methods of the compound. Although an enhancement of the antidiabetic effect of $VOSO_4$ is correlated with an increase in the plasma vanadium level, the development of toxicity by increasing the concentration of the metal ion should be avoided. For this purpose, we selected two different methods of administration. The first method uses the complexation of $VOSO_4$ to enhance the activity of the metal; chelation reduces the polarity of the metal and thus permeates the complex through the lipid layer of the cell membrane. The second method increases the bioavailability of $VOSO_4$ itself. For example, the capsulation of $VOSO_4$ allows delivering metal ions to the most desirable gastrointestinal sites, where $VOSO_4$ can be absorbed. We have already published reviews of the first method [8, 9]. In this review, we concentrate on a trial of the capsulation of $VOSO_4$, evaluated through a pharmacokinetic study of $VOSO_4$ and its complexes.

IN VIVO BLOOD CIRCULATION MONITORING-ELECTRON PARAMAGNETIC RESONANCE (BCM-EPR) METHOD

Recently, we proposed an *in vivo* BCM-EPR method to measure the real-time disposition of organic stable spin probes in the circulating blood of rats [16]. We then applied this method to the development of clinically useful reagents, and determined the systematic and quantitative pharmacokinetic features of insulinomimetic vanadyl compounds [17-20]. Vanadyl compounds were given by a

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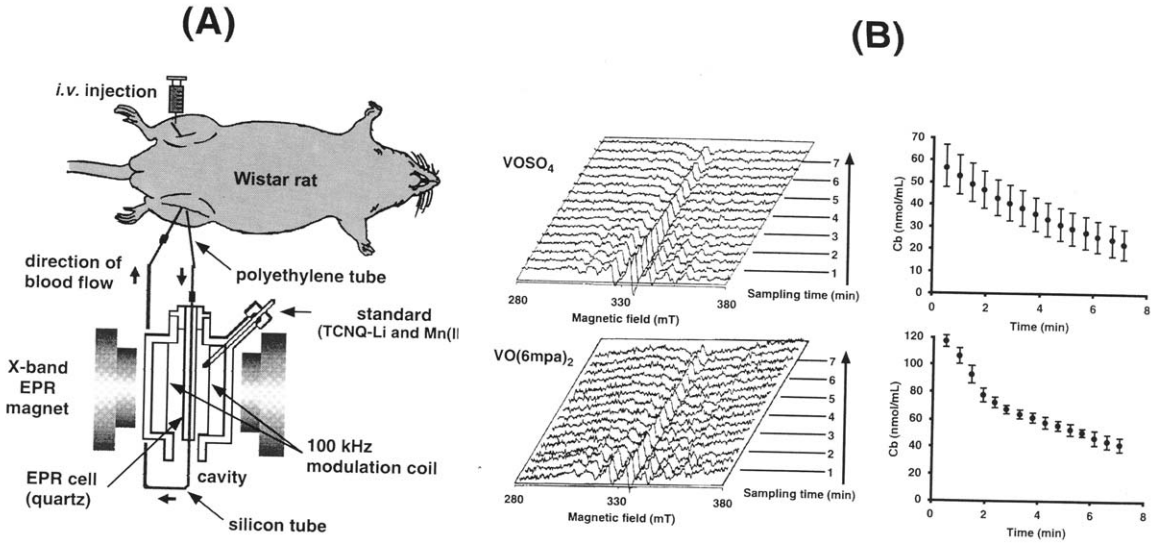

Fig. (1). *In Vivo* blood circulation monitoring-electron paramagnetic resonance (BCM-EPR) method (A) measuring system, and (B) EPR spectral changes in the blood of rats given $VOSO_4$ and $VO(6pma)_2$ complex by single intravenous injection. (References [16] and [17]).

single i.v. injection to rats at 37°C under pentobarbital anesthesia, and the EPR spectra were measured at room temperature every 30 seconds. The disappearance of the EPR signal due to paramagnetic vanadyl species (^{51}V: natural abundance 99.76%, nuclear spin I = 7 / 2) in the blood was plotted against time after the compounds were administered, and the data were analyzed by one or two compartment models (Fig. (**1**)). The EPR signals of the vanadyl compounds added to the fresh blood of untreated rats were very stable over time, indicating that a major factor in the disappearance of the EPR signals was not the redox processes of vanadyl species in the circulating blood, but rather the distribution of those species to the tissues and their elimination from the body.

We administered vanadyl sulfate ($VOSO_4$), bis(picolinato)oxovanadium(IV) ($VO(pic)_2$), and bis(6-methylpicolinato)oxovanadium(IV) ($VO(6mpa)_2$) (Fig. (**2**)) to healthy Wistar rats at a dose of 9.8 μmol (0.5mg) / kg body weight, and obtained the pharmacokinetic parameters

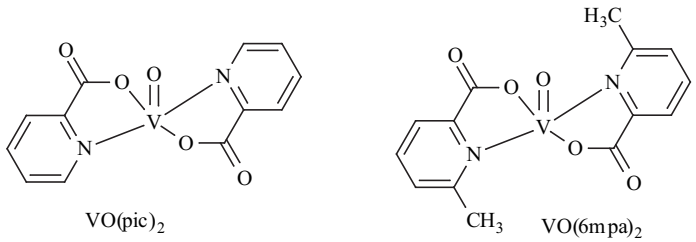

Fig. (2). Chemical structure of $VO(pic)_2$ and $VO(6pma)_2$ complexes.

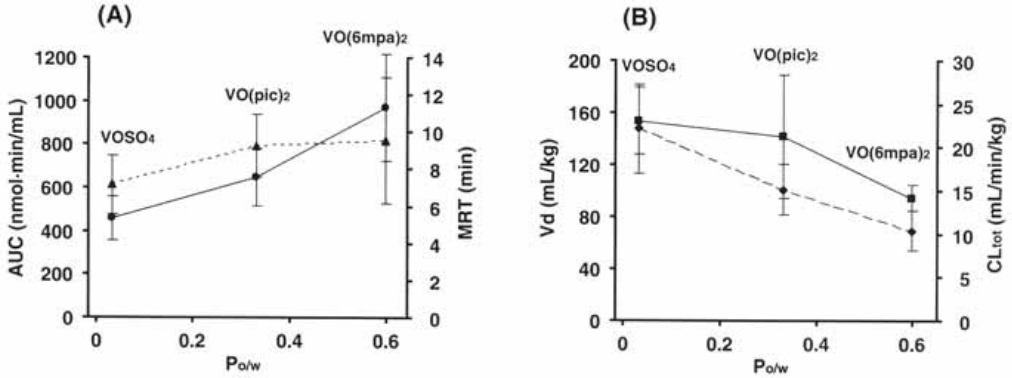

Fig. (3). Relationship between partition coefficients and pharmacokinetic parameters of vanadyl compounds (A) AUC (closed circle: •) and MRT (closed triangle: ▲) (B) Vd (closed square: ■) and CLtot (closed diamond: ♦). Partition coefficients were evaluated by *n*-octanol: Krebs ringer bicarbonate buffer (pH 7.4)= 1: 1 for 6 h. (References [17]).

Table 1. Pharmacokinetic Parameters of Vanadyl-Picolinate Complexes

	$t_{1/2}(\beta)$ (min)	Vd (mL/kg)	CL_{tot}(mL/min/kg)	AUC(μmol • min/mL)	MRT(min)
$VOSO_4$	3.0 ± 0.5	167 ± 10	38.9 ± 5.8	0.26 ± 0.04	4.4 ± 0.7
$VO(pic)_2$	8.2 ± 1.1**	142 ± 27	15.2 ± 2.9 **	0.65 ± 0.13**	9.3 ± 1.7 **
$VO(6mpa)_2$	14.8 ± 5.3 $^{**}_{\dagger}$	132 ± 14*	6.8 ± 1.5 $^{**}_{\dagger\dagger}$	1.45 ± 0.37 $^{**}_{\dagger\dagger}$	19.6 ± 6.9 $^{**}_{\dagger}$

Rats were treated with vanadyl complexes such as $VOSO_4$ (n=4), $VO(pic)_2$ (n=4), and $VO(6mpa)_2$ (n=3).
V : 9.8 μmol (0.5 mg)/kg body weight by intravenous injection under anaesthesia.
Significance level : * $p < 0.05$ and ** $p < 0.01$ vs $VOSO_4$
Significance level : † $p < 0.05$ and †† $p < 0.01$ vs $VO(pic)_2$
(Reference [20]).

of vanadyl species from time-dependent disappearance curves for three vanadyl compounds (Table (**1**)). We then examined the relationships between their parameters and their partition coefficients (Fig. (**3**)).

Vd (steady-state distribution volume in the body) of the hydrophobic $VO(6mpa)_2$ (95 mL / kg) was significantly lower than that of hydrophilic $VOSO_4$ (154 mL / kg). CL_{tot} (total body clearance) of $VO(6mpa)_2$ (10.5 mL / min / kg) was also significantly lower than that of $VOSO_4$ (22.2 mL / min / kg) and that of $VO(pic)_2$ (15.2 mL / min / kg). Therefore, *AUC* (area under the concentration curve) of $VO(6mpa)_2$ (974 nmol • min / mL) was significantly higher than that of $VOSO_4$ (461 nmol • min / mL) and that of $VO(pic)_2$ (651 nmol • min/mL), and *MRT* (mean residence time) of $VO(6mpa)_2$ (9.6 min) was slightly longer than that of $VOSO_4$ (7.2 min). Distribution to the tissues and elimination from the blood of $VO(6mpa)_2$, with a partition coefficient ($P_{o/w}$ = 0.60), apparently were lower in comparison to $VOSO_4$ ($P_{o/w}$ = 0.03) and $VO(pic)_2$ ($P_{o/w}$ = 0.33), and the exposure amounts of $VO(6mpa)_2$ in the blood space were approximately 1.5-2 times higher than those of $VOSO_4$ and $VO(pic)_2$. This result is in close agreement with the high hypoglycemic activity of $VO(6mpa)_2$ compared with $VOSO_4$ and $VO(pic)_2$ [20].

In general, the tissue distribution of a compound increases as its hydrophobicity increases. However, the results of this study were unusual. It has been reported that the vanadate ion is incorporated into cells through non-specific anion channels [21]. In general, the metal ions bind with serum albumin. For example, BSA (Bovine seroalbumin) was found to have 40 binding sites to vanadium ions [22]. However, when a metal-ligand complex is stable in biological fluids, its protein binding ability and its transport processes from blood into tissues may alter its pharmacokinetic features compared with those of the free form of the metal ion. Actually, the clearance curve of vanadyl species from the blood in rats given $VOSO_4$ was analyzed with the one-compartment model, while those in rats given $VO(pic)_2$ or $VO(6mpa)_2$ were analyzed with the two-compartment model. Thus, the transfer rates of vanadyl ion ($VOSO_4$) between the central and peripheral compartments were considered to be very fast and the distribution processes of $VOSO_4$ between the blood and the tissues achieved a rapid equilibrium condition, in comparison with vanadyl complexes ($VO(pic)_2$ and $VO(6mpa)_2$). Therefore, we speculated that $VO(6mpa)_2$, with a higher hydrophobicity than $VO(pic)_2$ and $VOSO_4$ ($P_{o/w}$ values: $VO(6mpa)_2$ (0.60) > $VO(pic)_2$ (0.33) > $VOSO_4$ (0.03)), is not easily transferred from blood to tissues in comparison with the hydrophilic $VOSO_4$.

These characteristics of $VO(pic)_2$ and $VO(6mpa)_2$ differ from those of $VOSO_4$ and indicate that the hypoglycemic activity of the two compounds is long-term, lasting even after the compound administration to the STZ-induced type 1 diabetic rats ceased [23].

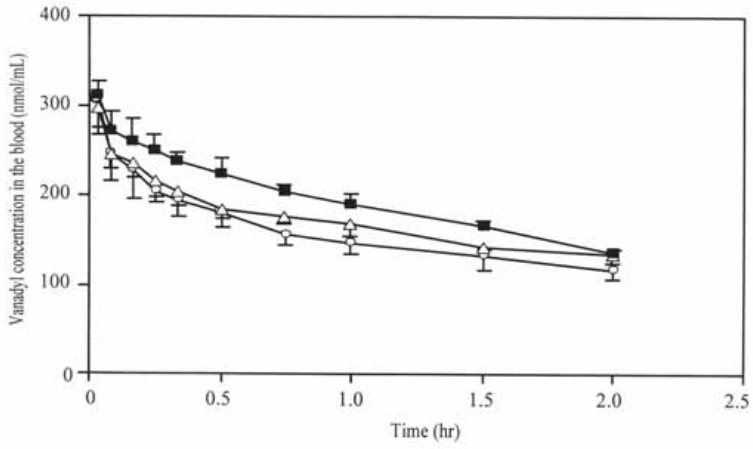

Fig. (4). Time course for vanadyl concentration in the blood of rats after intravenous administration. Rats were given $VOSO_4$ (open circle: O), VO $(pic)_2$ (open triangle: Δ), or $VO(6mpa)_2$ (closed square: ■) at a dose of 39 μmol (2 mg) / kg body weight. Each symbol represents the mean ± S.D. for 3 rats. (References [24]).

Table 2. Pharmacokinetic Parameters in the Absorption and Elimination Processes of $VOSO_4$, $VO(pic)_2$, and $VO(6mpa)_2$ after Intravenous (i.v.) and Intraperitoneal (i.p.) Administration

Administration route	Dose (μmol/kg)	Compound	AUC (nmol hr/mL)	C_{max} (nmol/mL)	MRT (hr)	T_{max} (hr)	CL_{tot} (mL/kg • hr)	Vd (mL/kg)	Fa (%)
i.v.	39	$VOSO_4$	697 ± 89		3.28 ± 0.76		56.9 ± 7.7	183 ± 22	
		$VO(pic)_2$	845 ± 59		3.80 ± 0.43		46.6 ± 3.3	176 ± 8	
		$VO(6mpa)_2$	792 ± 16		2.90 ± 0.12		49.5 ± 1.0	144 ± 3	
i.p.	196	$VOSO_4$	1573 ± 109	203 ± 29	3.28 ± 0.76	2.00 ± 0.00			45.6
		$VO(pic)_2$	3484 ± 284*	567 ± 47 **	3.80 ± 0.43*	2.33 ± 0.58			82.8
		$VO(6mpa)_2$	4248 ± 300 *, †	365 ± 34 **, †	2.90 ± 0.12 ††	3.00 ± 1.00			107.2

Data are shown as the mean ± S.D. for 3 rats.
Significance level: * $p<0.05$ and ** $p<0.01$ vs $VOSO_4$ † $p<0.05$ and †† $p<0.01$ vs $VO(pic)_2$
(Reference [24]).

GASTROINTESTINAL ABSORPTION FEATURES OF VANADYL COMPOUNDS

Following the short-term pharmacokinetic study of vanadyl compounds by the BCM-EPR method, we used EPR spectrometry in a long-term pharmacokinetic study of the compounds. We examined the absorption processes of the gastrointestinal tract of healthy rats given bolus $VOSO_4$, $VO(pic)_2$, and $VO(6mpa)_2$ compounds [24].

First, $VOSO_4$, $VO(pic)_2$, or $VO(6mpa)_2$ was injected intravenously to rats at a dose of 39 μmol (2 mg) / kg body weight. The time course of vanadyl concentrations in the blood is shown in Fig. (**4**), and the pharmacokinetic parameters obtained from the concentration curves are summarized in Table (**2**). The pharmacokinetic parameters of the three vanadyl compounds did not differ significantly with each other after the intravenous administration.

Other rats were then given one of the three compounds by intraperitoneal injection, the administered dose being fixed at 196 μmol (10 mg) / kg body weight. CL_{tot} values obtained from the intravenous data were used for calculating the bioavailability *Fa* value, which is defined as an absorption ratio from non-intravenous routes. The time course of vanadyl concentration in the blood and the pharmacokinetic parameters obtained are shown in Fig. (**5**) and Table (**2**), respectively.

T_{max} values were almost the same for the three compounds, whereas, for an extended period (3-16 hr), a higher vanadyl concentration in the blood of rats treated with $VO(6mpa)_2$ was observed, indicating that the behavior of $VO(6mpa)_2$ in the absorption process from the abdominal cavity to the systemic circulation differs remarkably from the behaviors of $VOSO_4$ and $VO(pic)_2$. *Fa* values of rats treated with $VOSO_4$, $VO(pic)_2$, and $VO(6mpa)_2$ were 45.6, 82.8, and 107.2%, respectively. C_{max} values increased in the following order: $VO(pic)_2$ (567 ± 47 nmol / mL) > $VO(6mpa)_2$ (365 ± 34 nmol / mL) > $VOSO_4$ (203 ± 29 nmol / mL). *MRT* values of $VOSO_4$ and $VO(6mpa)_2$ were almost the same (7.89 ± 0.22 hr and 7.99 ± 0.17 hr, respectively), whereas that of $VO(pic)_2$ was the shortest (5.25 ± 0.17 hr) (Table (**2**)). Previously, we reported that a low dose of $VO(6mpa)_2$ normalizes the glucose levels of

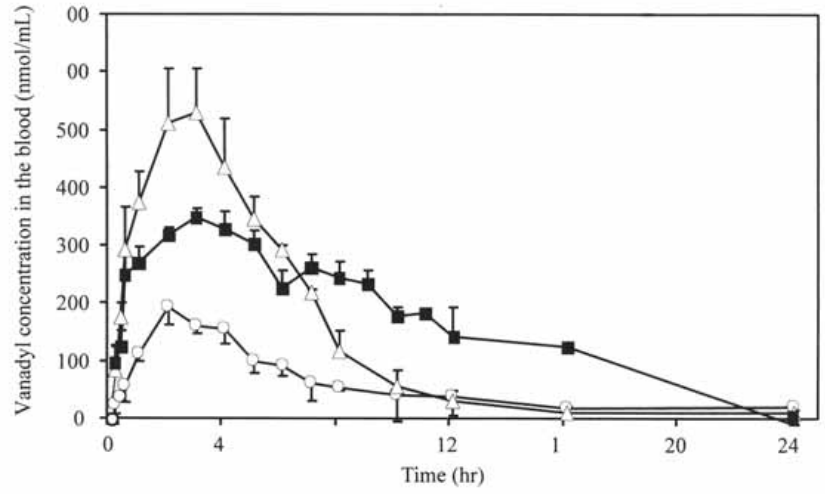

Fig. (5). Time course for vanadyl concentration in the blood of rats after intraperitoneal administration. Rats were given $VOSO_4$ (open circle: O), $VO(pic)_2$ (open triangle: Δ), or $VO(6mpa)_2$ (closed square: ■) at a dose of 196 μmol (10 mg) / kg body weight. Each symbol represents the mean ± S.D. for 3 rats. (References [24]).

STZ-rats after intraperitoneal administration [24], suggesting that vanadyl species, after intraperitoneal administration of $VO(6mpa)_2$, distribute and accumulate in the body and the blood more efficiently than do other compounds. The extended periods of higher blood concentrations of vanadyl species after $VO(6mpa)_2$ administration indicated that $VO(6mpa)_2$ treated type 1 DM of STZ-rats at lower doses than did $VOSO_4$ and $VO(pic)_2$ [24].

On the basis of these results, the intestinal absorption profiles of vanadyl compounds were examined after oral administration (Fig. (**6**) and Table (**3**)).

Fa values of rats given $VOSO_4$, $VO(pic)_2$ and $VO(6mpa)_2$ were estimated to be 4.8, 5.3, and 9.8%, respectively. C_{max} of $VO(6mpa)_2$ was significantly higher (54.6 ± 9.7 nmol / mL) than that of $VO(pic)_2$ (26.2 ± 3.4 nmol / mL) and that of $VOSO_4$ (18.9 ± 5.2 nmol / mL) (Table (**2**)). *MRT* values of the three vanadyl compounds were almost the same, whereas T_{max} of $VO(6mpa)_2$ was the earliest (5.50 ± 0.58 hr), followed by $VO(pic)_2$ (7.00 ± 1.00 hr) and $VOSO_4$ (8.33 ± 3.79 hr). It is quite interesting to note that two absorption maxima were observed in the vanadyl concentration curves of $VOSO_4$ and $VO(pic)_2$, while a single absorption maximum was found in that of $VO(6mpa)_2$ (Fig. (**5**)). T_{max} values for the first maximum of $VOSO_4$ and $VO(pic)_2$ were almost the same (4 hr), though T_{max} for the second maximum of $VO(pic)_2$ was significantly earlier than that of $VOSO_4$. These results suggested that the

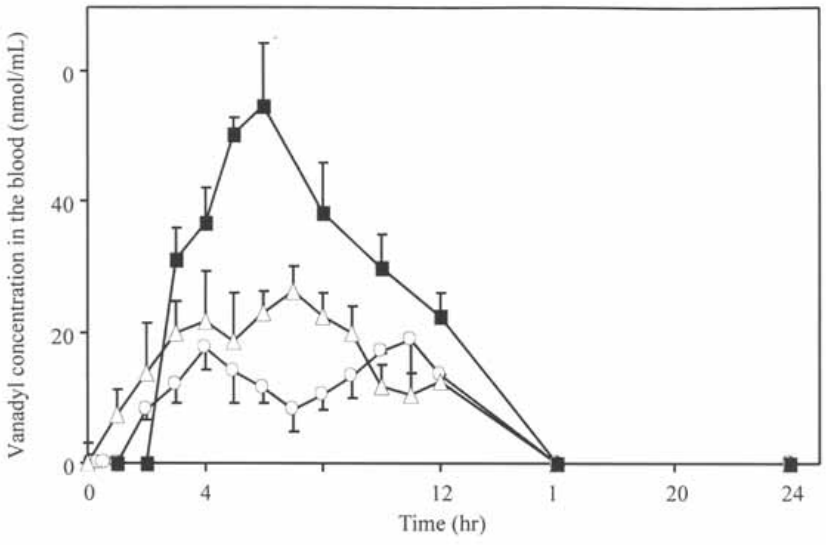

Fig. (6). Time course for vanadyl concentration in the blood of rats after oral administration. Rats were given $VOSO_4$ (open circle: O), $VO(pic)_2$ (open triangle: Δ), or $VO(6mpa)_2$ (closed square: ■) at a dose of 196 μmol (10 mg) / kg body weight. Each symbol represents the mean ± S.D. for 3 or 4 rats. (References [24]).

Table 3. Pharmacokinetic Parameters in the Absorption Processes of $VOSO_4$, $VO(pic)_2$, and $VO(6mpa)_2$ after Oral, Intra-jejunal and Intra-ileal Administration

Compound	Administration site	AUC (nmol • hr/mL)	C_{max} (nmol/mL)	MRT (hr)	T_{max} (hr)	Fa (%)	Enhancement of Fa
$VOSO_4$	stomach	165 ± 6	18.9 ± 5.2	7.93 ± 0.12	8.33 ± 3.79	4.8	1
	jejunum	348 ± 5	47.8 ± 7.4	5.20 ± 1.53	2.67 ± 1.53	10.1	2.11
	ileum	433 ± 57	31.5 ± 12.9	9.09 ± 0.41	1.50 ± 0.87	12.6	2.62
$VO(pic)_2$	stomach	223 ± 12	26.2 ± 3.4*	6.87 ± 0.09 *	7.00 ± 1.00	5.3	1
	jejunum	442 ± 23	45.2 ± 1.2	8.13 ± 0.28 *	4.67 ± 0.58	10.5	1.98
	ileum	454 ± 43	56.6 ± 8.3*	7.44 ± 0.57*	4.00 ± 1.73	10.8	2.04
$VO(6mpa)_2$	stomach	388 ± 52 *, †	54.6 ± 9.7 *, †	7.60 ± 0.20†	5.50 ± 0.58	9.8	1
	jejunum	311 ± 65†	53.0 ± 3.6	7.30 ± 1.00*	6.50 ± 0.58 *, †	7.8	0.80
	ileum	699 ± 9 **, †	99.3 ± 4.9 **, ††	6.82 ± 0.11**	5.00 ± 0.00 **	17.6	1.80

Data are shown as the mean values ± S.D. for 3 or 4 rats

Rats were given $VOSO_4$ $VO(pic)_2$, or $VO(6mpa)_2$ at a does of 196 μmol (10 mg) / kg body weight.

Significance level: * $p<0.05$ and ** $p<0.01$ vs $VOSO_4$ at the same administration site, † $p<0.05$ and †† $p<0.01$ vs $VO(pic)_2$ at the same administration site

Enhancement of Fa is calculated as [Fa value of rats given each compound after each administration sites / that aftter oral administration].

(Reference [24]).

gastrointestinal tract contains multiple absorption sites for these compounds. When $VO(6mpa)_2$ suspended in saline containing 5% acacia was orally given to rats, the complex was shown to resolve in the intestinal lumen before the absorption into 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 the absorption of vanadyl compounds in terms of *Fa* values agreed closely with those from a previous study in rats [25].

Then, we examined the absorption of the vanadyl compounds after intra-jejunal administration (Fig. (**7**) and Table (**3**)). *Fa* and C_{max} values of rats were almost the same among the three compounds (*Fa* value: $VOSO_4$ 10.1%, $VO(pic)_2$ 10.5%, and $VO(6mpa)_2$ 7.8%; C_{max} value: $VOSO_4$ 47.8 ± 7.4 nmol / mL, $VO(pic)_2$ 45.2 ± 1.2 nmol / mL, and $VO(6mpa)_2$ 53.0 ± 3.6 nmol / mL. MRT of $VOSO_4$ was significantly shorter (5.20 ± 0.03 hr) than that of $VO(6mpa)_2$ (7.30 ± 1.00 hr) and that of $VO(pic)_2$ (8.13 ± 0.28 hr). Similarly, the first T_{max} (2.67 ± 1.53 hr) of $VOSO_4$ was significantly shorter than that of $VO(pic)_2$ (4.67 ± 0.58 hr) and that of $VO(6mpa)_2$ (6.50 ± 0.58 hr). Two absorption maxima were observed in each compound's concentration curve, and the second T_{max} of $VOSO_4$ (5 hr) was earlier than that of $VO(pic)_2$ and that of $VO(6mpa)_2$ (7 hr).

Finally, the absorption of vanadyl compounds was examined after intra-ileal administration (Fig. (**8**) and Table (**3**)). *Fa* value of rats given $VO(6mpa)_2$ intra-ileally was larger (17.6%) than that of rats given either of the other

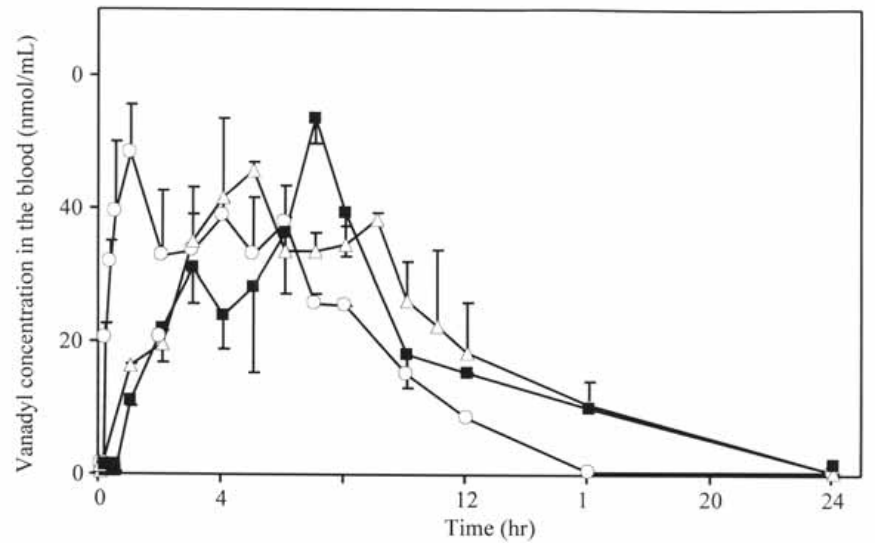

Fig. (7). Time course for vanadyl concentration in the blood of rats after intra-jejunal administration. Rats were given $VOSO_4$ (open circle: O), $VO(pic)_2$ (open triangle: Δ), or $VO(6mpa)_2$ (closed square: ■) at a dose of 196 μmol (10 mg) / kg body weight. Each symbol represents the mean ± S.D. for 3 or 4 rats. (References [24]).

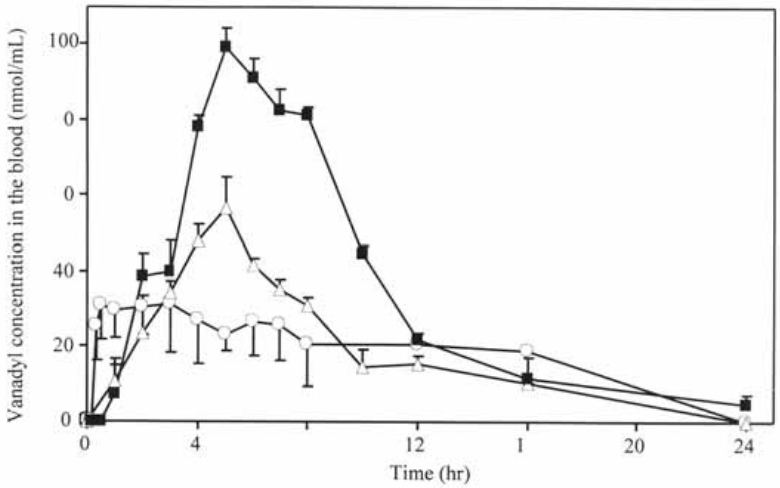

Fig. (8). Time course for vanadyl concentration in the blood of rats after intra-ileal administration. Rats were given $VOSO_4$ (open circle: O), $VO(pic)_2$ (open triangle: Δ), or $VO(6mpa)_2$ (closed square: ■) at a dose of 196 μmol (10 mg) / kg body weight. Each symbol represents the mean ± S.D. for 3 or 4 rats. (References [24]).

compounds. In addition, C_{max} was significantly higher in $VO(6mpa)_2$ (99.3 ± 4.9 nmol / mL) than in the other compounds. *MRT* of $VOSO_4$ after intra-ileal administration was longer (9.09 ± 0.41 hr) than that of $VO(pic)_2$ (7.44 ± 0.57 hr) and that of $VO(6mpa)_2$ (6.82 ± 0.11 hr) (Table (**3**)), whereas T_{max} of $VOSO_4$ occurred the earliest (1.50 ± 0.87 hr) of the three. C_{max} and *Fa* values of the three compounds were approximately twice as high after intra-ileal administration than after oral administration. When any of the three compounds was administered directly into the large intestine, the concentration of vanadyl species in the blood was below the detection limit. This lack of absorption from the large intestine suggested that absorption from the ileum was responsible for the single absorption maximum observed after intra-ileal administration.

After oral administration, the vanadyl complexes are supposed to decompose mostly in the stomach, where the pH is 1.5-3.5 [26]. 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 a higher level of pharmacological activity.

Based on the above results, we proposed that dosage delivered to the ileum in an intact form will improve the bioavailability of vanadyl complexes and, in turn, will increase the insulinomimetic activity of the vanadyl complexes after oral administration.

TRIAL FOR THE PREPARATION OF ENTERIC-COATED CAPSULES CONTAINING VANADYL SULFATE

After we found that $VOSO_4$ is absorbed more thoroughly at the ileum than at other gastrointestinal sites, we investigated the absorption process following oral administration of $VOSO_4$ by enteric-coated capsules (ECC) [27].

Mini gelatin capsules (GC) (diameter 2.5 mm, length 8.5 mm) were filled with solid $VOSO_4$ at a dose of 196 μmol (10 mg) / kg body weight by using a miniature-capsule filling device. After $VOSO_4$-containing mini GC were placed in a hydroxypropylmethyl-cellulose phthalate (HPMCP) solution (solvent: methylene chloride / methanol = 4 / 1), the solvent was evaporated at 4°C and thus $VOSO_4$-containing ECC were prepared.

The ECC were administered to healthy rats by single oral administration, and the time courses of vanadyl concentration in the blood were examined by using EPR. The data for ECC were compared with the data for GC. As shown in Fig. (**9**), the delayed appearance of vanadyl concentration after oral administration of ECC was attributed to the delayed release of $VOSO_4$ compared with the release from GC. For 8 to 12 hr after oral administration of ECC, the vanadyl concentration was maintained at higher levels

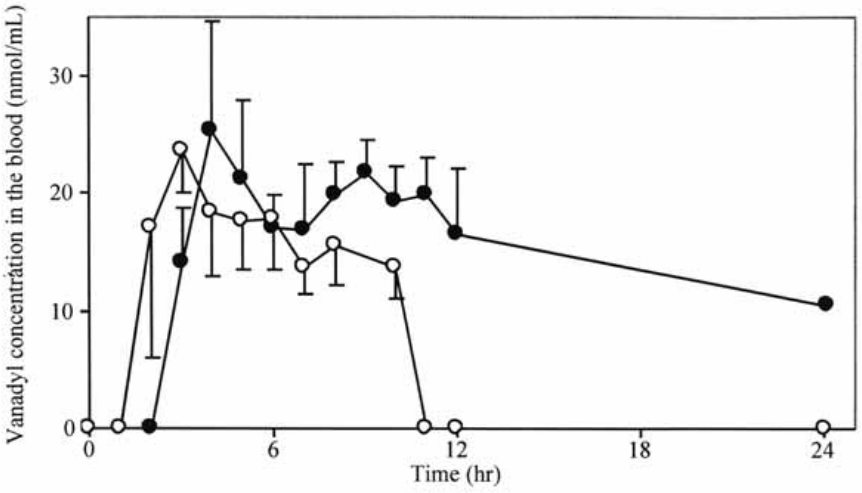

Fig. (9). Time course for vanadyl concentration in the blood of rats after oral administration of gelatin capsules (open circle: O) or enteric-coated capsules (closed circle: •) containing $VOSO_4$ at a dose of 196 μmol (10 mg) / kg body weight. Each symbol represents the mean ± S.D. for 4 rats. (References [27]).

Table 4. Pharmacokinetic Parameters for Vanadyl Species in the Blood of Rats after Oral Administration of $VOSO_4$ in Rats

Form of administration	AUC (nmol • hr/mL)	C_{max} (nmol/mL)	MRT (hr)	T_{max} (hr)	Fa (%)	MAT (hr)
Geletin capsule	137 ± 37	24.5 ± 4.6	5.38 ± 0.27	2.75 ± 0.50	4.0	2.10
Enteric-coated capsule	338 ± 46 **	27.2 ± 7.4	11.73 ± 0.49 **	4.75 ± 0.96 *	9.8	8.45

Rats were given $VOSO_4$ at a does of 196 μmol (10 mg) / kg body weight.
Data are shown as the mean ± S.D. for 4 rats
Significance level: * $p<0.05$ and ** $p<0.01$ vs gelatin capsule
(Reference [27]).

than that observed with GC. This difference was observed at up to 24 hr after administration, indicating that ECC passed through the stomach without disintegrating and released $VOSO_4$ into the small intestine, where the $VOSO_4$ was absorbed.

We then obtained the pharmacokinetic parameters to evaluate the absorption process and bioavailability of $VOSO_4$ after different means of administration (Tables (**3**) and (**4**)). C_{max} values of vanadyl species were similar for the two forms of $VOSO_4$ (ECC: 24.5 ± 4.6 nmol / mL and GC: 27.2 ± 7.4 nmol / mL) and were at almost the same level as that for the solution form (18.9 ± 5.2 nmol / mL), whereas *AUC* of vanadyl species during 24 hr was significantly larger with ECC (338 ± 46 nmol • hr / mL) than with either GC (137 ± 37 nmol • hr / mL) or the solution (165 ± 6 nmol • hr / mL). *Fa* of the vanadyl species for ECC (9.8%) increased almost twice as much as that associated with either GC (4.0%) or the solution (4.8%).

Both T_{max} and *MRT* values of vanadyl species were significantly longer for ECC than for GC. This difference is probably owing to a number of factors, including differences in the transit of the capsules through the gastrointestinal tract, disintegration of the capsules at a site in the intestine with the appropriate pH level, dissolution of solid $VOSO_4$ in the lumen, and absorption of $VOSO_4$ at different sites along the gastrointestinal tract [28].

MAT of vanadyl species for ECC (8.4 hr) was approximately four times greater than that for GC (2.1 hr, Table (**4**)). This difference is attributable to the transit of ECC in the lumen of the gut and also to the slower absorption rate of vanadyl species at the ileum. In addition, *MAT* of vanadyl species after intra-ileal administration of $VOSO_4$ solution (5.8 hr [24]) was much longer than *MRT* after intravenous administration (3.3 hr [24]), suggesting that the absorption rate constant of vanadyl species is smaller than the elimination rate constant for these species [29].

From these results, it is clear that administration of ECC containing $VOSO_4$ improved vanadyl absorption more than either GC or the solution did. We propose that administration of enteric-coating capsulation of $VOSO_4$ is an effective means to enhance the bioavailability of $VOSO_4$.

We are now testing whether or not ECC containing $VOSO_4$ at half-doses of GC or solution administration normalizes the hyperglycemia of STZ-rats. Likewise, we are preparing ECC containing vanadyl complexes such as $VO(pic)_2$ and $VO(6mpa)_2$, which may enable reduced dosages to exhibit the same degree of blood glucose lowering effects as that provided by GC or solution administration.

CONCLUSION

On the basis of our results in experimental animals, oral administration of vanadyl compounds has been found to be the only effective way to replace insulin injections. In this article, we proposed two methods for the oral administration of vanadyl compounds, involving complexation and capsulation of vanadyl ion. A large number of people, especially young people suffering from type 1 diabetes mellitus, are expecting a simple and painless therapeutic method to treat the disease. We hope that this article will aid in the development of new therapeutic methods in the future.

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