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Enteric-coating capsulation of insulinomimetic vanadyl sulfate enhances bioavailability of vanadyl species in rats

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Abstract

In recent years, there have been improvements in the treatment of type 2 diabetes by oral administration of vanadyl sulfate ($VOSO_4$, VS). The maintenance of vanadyl levels in the blood of subjects with type 2 diabetes was found to be important for the insulinomimetic activity of VS. However, owing to low bioavailability of VS and the development of mild gastrointestinal symptoms and side-effects in some subjects, it is necessary to design more effective and safer dosages of VS. After discovering that VS is absorbed more thoroughly at the ileum than at other gastrointestinal sites, we investigated the absorption processes following oral administration of VS by preparing enteric-coated capsules (ECC). Although C_{max} values were unchanged by the dosage forms, T_{max} and MRT values associated with the enteric-coating capsulation were prolonged when compared with those observed with use of gelatin capsules (GC). An important finding was that the bioavailability of VS from ECC (9.8%) was almost double that of VS from either GC (4.0%) or the solution (4.8%). Administration of VS-containing ECC to diabetic patients is proposed to improve vanadyl absorption over that achieved by the administration of either GC or the solution.

Introduction

Vanadium compounds are known to show insulinomimetic effects in in-vitro and in-vivo systems (Shechter & Shisheva 1993; Badmaev et al 1999; Thompson 1999; Sakurai et al 2002). Because vanadyl ion (+4 oxidation state of vanadium) is less toxic than vanadate (+5 oxidation state of vanadium) (Hudson 1964) and the predominant form in rats (Sakurai et al 1980, 1990), this ion is thought to be the active intracellular form exhibiting insulinomimetic activity (Sakurai et al 1990; Shechter & Shisheva 1993; Thompson 1999).

Recently, vanadyl sulfate ($VOSO_4$, VS) was shown to be advantageous in subjects with type 2 diabetes mellitus in terms of plasma glucose, haemoglobin A_{1c} (HbA_{1c}) and fructosamine levels, when given orally at a daily dose of 150 mg for 6 weeks (Cusi et al 2001). Plasma vanadium concentrations were below 10 $\mu g\ L^{-1}$ before treatment, but were increased to $104 \pm 18\ \mu g\ L^{-1}$ after 6 weeks of VS administration, as analysed by atomic absorption spectroscopy. These results indicate the importance of plasma vanadium concentrations for antidiabetic activity during VS treatment.

Recently, we found that the bioavailability of VS following a bolus oral administration in healthy rats was 4.8%, with the active form of vanadyl species in

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the blood determined by electron spin resonance spectroscopy (ESR) (Fugono et al 2001). However, the bioavailability of vanadyl species was enhanced approximately two- and threefold when VS was administered into the jejunum and ileum, respectively. These results prompted us to examine whether VS should be administered directly into the jejunum and ileum of rats. For this purpose, enteric-coated capsules (ECC) containing solid VS were prepared and administered to rats, and blood levels of vanadyl species were monitored by ESR.

Materials and Methods

Materials

Mini gelatin capsules (GC) were purchased from Shionogi Qualicaps Co. (Nara, Japan). Hydroxypropylmethylcellulose phthalate (HPMCP, HP-55 grade) was a kind gift from Shin-Etsu Chemical Industrial Co. (Tokyo, Japan). Vanadyl sulfate ($VOSO_4 \cdot 2 \cdot 3H_2O$, VS) was purchased from Wako Pure Chemicals Co. (Osaka, Japan), and its purity was determined by chelatometry (Fugono et al 2001). All other materials used were of analytical reagent grade.

Animals

Male Wistar rats (8 weeks old), 240–250 g, (Simizu Experimental Material Co., Kyoto, Japan) were housed in a temperature-controlled room at 23 ± 1°C, fed a standard laboratory diet, and given water *ad libitum*. They were fasted overnight for 12 h before the experiments. All animal experiments were approved by the Experimental Animal Research Committee of Kyoto Pharmaceutical University and were performed according to the Guidelines for Animal Experimentation of Kyoto Pharmaceutical University.

Preparation of VS-containing GC

Mini GC (diameter 1.5 mm, length 8.0 mm) were used for the oral administration of VS. They were filled with solid VS at a dose of 10 mg (196 μmol) vanadium kg^{-1} bodyweight by using a miniature capsule filling device (Shionogi Qualicaps Co.).

Preparation of VS-containing ECC

HPMCP solution (40 g L^{-1}) was prepared in a mixture of methylene chloride and methanol (4:1). After VS-containing mini GC were placed in the mixture, the solvent was evaporated overnight at 4°C in a refrigerator. The capsules were kept in a desiccator on silica gel until use.

Oral administration of VS-containing GC and ECC in rats

Before administration, a 200-μL blood sample was collected from the jugular vein of rats under light ether anaesthesia by puncture with a heparinized syringe with a 26-G needle to prepare the calibration curves of vanadyl species in the blood. One GC or ECC containing VS at a dose of 10 mg (196 μmol) vanadium kg^{-1} was orally administered to rats through a miniature capsule administration device (Shionogi Qualicaps Co.) conjoined to a 1-mL disposable syringe containing 0.5 mL physiological saline (0.9% NaCl). After administration, blood samples were periodically collected for 36 h from the jugular vein of rats.

ESR determination of vanadyl species in the blood of rats treated with VS-containing GC or ECC

ESR spectra of vanadyl species in the blood were measured with a JES-RE1X spectrometer (Jeol, Tokyo, Japan) at room temperature (25°C) under the following conditions: field modulation frequency 100 kHz, modulation amplitude width 2.0 mT, microwave power 5.0 mW, magnetic field 335 ± 50 mT, sweep time 1 min, and response of 0.03 s. A quartz capillary (i.d. 0.5 mm, length 10 cm) (Eiko-sha, Osaka, Japan) was used for the measurement of blood samples. Storage and analysis of the ESR data were performed with an ESR data analyser (Radical Research, Tokyo, Japan).

The blood samples, kept at 4°C, were measured on the same day as blood sampling. An aliquot (20 μL) of each blood sample was transferred to a quartz capillary, which was then inserted in a quartz ESR measuring tube (i.d. 4 mm) and fixed in the ESR cavity.

To determine the concentrations of vanadyl species in the blood, various concentrations of VS were added to the fresh blood of untreated rats. ESR measurements were performed as described above. A calibration curve was obtained by monitoring signal intensities at a central signal in the 8-line spectrum of vanadyl species. A linear relationship between ESR signal intensities and four different concentrations of VS was demonstrated over the range of 25–100 nmol mL^{-1}, with a linear correlation coefficient greater than 0.999 for four concentrations of VS in three repeated measurements. The detection limit at an S/N ratio of 3 was 10 nmol mL^{-1} in blood.

Metallokinetic analysis of vanadyl species in the blood of rats

Time courses of vanadyl concentrations in blood were evaluated on the basis of non-compartmental pharmacokinetic analysis (Yamaoka et al 1978). The area under the vanadyl blood concentration–time curve (AUC), maximal vanadyl concentration (C_{max}), mean residence time (MRT), and time required to attain C_{max} (T_{max}) for 24 h were estimated model-independently. Total clearance (CL) and the MRT after intravenous administration ($MRT_{i.v.}$) of VS were 56.9 ± 7.7 mL h^{-1} kg^{-1} and 3.28 ± 0.76 h, respectively (Fugono et al 2001). The absorption ratio (Fa, extent of bioavailability) in each group was calculated by the following equation: $Fa = AUC \cdot CL\ dose^{-1}$. Mean absorption time (MAT, rate of bioavailability) in each group was calculated as follows: $MAT = MRT - MRT_{i.v.}$.

Statistical analysis

All results were expressed as the arithmetic mean $\pm$ s.d. for four rats. The statistical analysis was performed using Student's *t*-test at 5% ($P < 0.05$) or 1% ($P < 0.01$) significance levels.

Results and Discussion

Oral administration of VS has been clinically tested to treat diabetic subjects (Cohen et al 1995; Boden et al 1996; Halberstam et al 1996; Goldfine et al 2000; Cusi et al 2001). The study by Cusi et al (2001) demonstrated an improvement of glycaemia and insulin sensitivity by decreasing the levels of plasma glucose and HbA_{1c}. However, mild gastrointestinal symptoms and side-effects such as nausea, mild diarrhoea and abdominal cramps were observed in some subjects during treatment. In order to achieve a safer and more effective treatment for diabetes mellitus, a controlled dosing regimen and a dosage standard for the oral administration of VS need to be established. Recently, we reported that VS was absorbed more thoroughly at the ileum than at other gastrointestinal sites, suggesting that an ECC delivered to the intestines and disintegrated there improves the bioavailability of VS (Fugono et al 2001) in terms of an increase in AUC and prolongation of T_{max} and MRT. We therefore developed an ECC in order to enhance the intestinal absorption of VS after oral administration.

The time courses of vanadyl concentration in blood following oral administration of VS in the form of either GC or ECC in rats are shown in Figure 1. Delayed appearance of vanadyl concentration after oral administration of ECC is owing to the delayed release of VS compared with that from GC. Vanadyl concentration after oral administration of ECC was maintained at higher levels than that observed with GC during 8–12 h; these effects were observed up to 24 h, indicating that ECC pass through the stomach without disintegration and release VS into the small intestine, where VS is absorbed. We evaluated the absorption process and bioavailability of VS administered in different forms and the obtained metallokinetic parameters are summarized in Table 1.

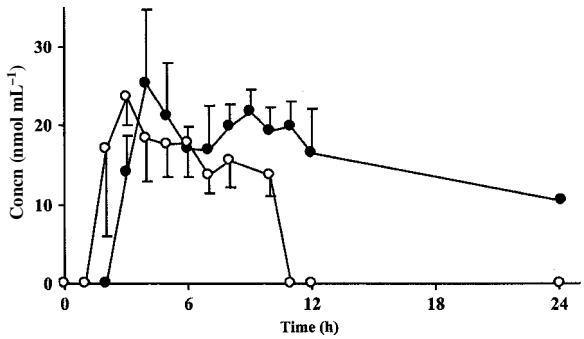

Figure 1 Time course for vanadyl concentration in blood after oral administration of gelatin capsules (○) or enteric-coated capsules (●) containing vanadyl sulfate at a dose of 10 mg vanadium kg^{-1} in rats. Each symbol represents the mean $\pm$ s.d., n = 4.

The C_{max} values of vanadyl species were similar for the two forms of VS and were at almost the same level as that for the solution form (18.9 ± 5.2 nmol mL^{-1}; Fugono et al 2001), whereas the AUC of vanadyl species during 24 h was significantly larger in the case of ECC than that of either GC or the solution (165 ± 6 nmol h mL^{-1}; Fugono et al 2001); the Fa of the vanadyl species for ECC (9.8%) increased almost two-fold compared with that associated with either GC (4.0%) or the solution (4.8%; Fugono et al 2001).

Both T_{max} and MRT values of vanadyl species for ECC were significantly longer than those 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 VS in the lumen, and absorption of VS at different sites along the gastrointestinal tract (Tanigawara et al 1982).

The MAT of vanadyl species for ECC (8.4 h) was approximately four times greater than that associated with GC (2.1 h; Table 1). This difference is attributable to the

Table 1 Metallokinetic parameters for vanadyl species in blood after oral administration of gelatin or enteric-coated capsules containing vanadyl sulfate in rats.

Form of administration	AUC (nmol h mL^{-1})	C_{max} (nmol mL^{-1})	MRT (h)	T_{max} (h)	Fa (%)	MAT (h)
Gelatin 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

AUC, area under the vanadyl blood concentration–time curve; C_{max}, maximal vanadyl concentration; MRT, mean residence time of vanadyl species; T_{max}, time required to attain C_{max}; Fa, absorption ratio; MAT, mean absorption time. AUC and MRT were estimated by trapezoidal integration for 0–24 h with extrapolation. Data are mean±s.d., n = 4. *$P < 0.05$, **$P < 0.01$ vs gelatin capsule.

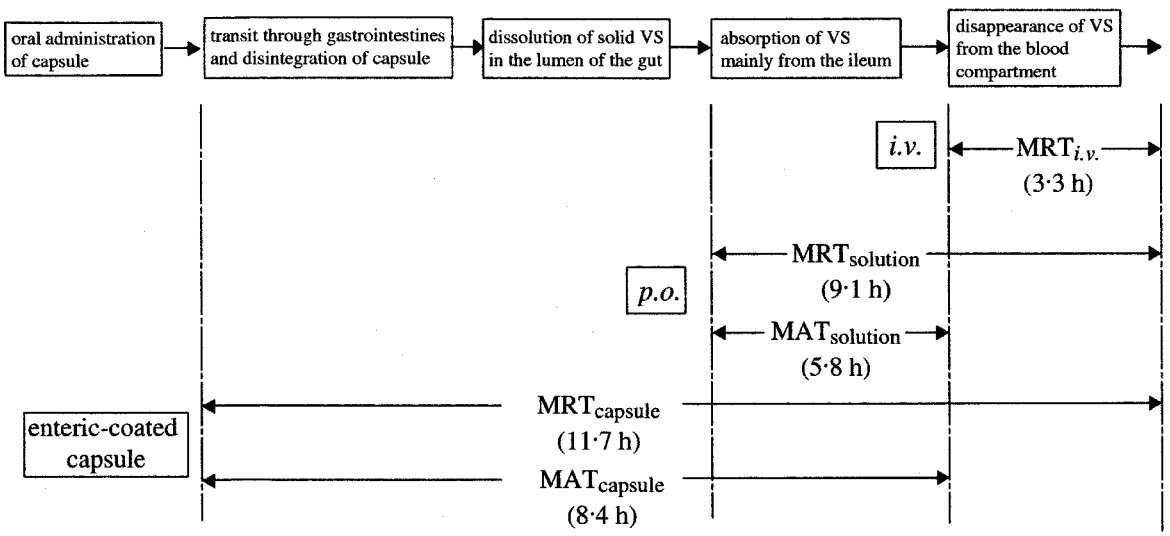

Figure 2 Schematic representation of the process of vanadyl sulfate (VS) absorption after oral administration of VS-containing enteric-coated capsules, oral administration of VS solution and intravenous administration of VS solution. MRT, mean residence time; MAT, mean absorption time.

transit of ECC in the lumen of the gut and also to the slower absorption rate of vanadyl species at the ileum. In addition, the MAT of vanadyl species after intra-ileal administration of VS solution (5.8 h; Fugono et al 2001) was much longer than the MRT after intravenous administration (3.3 h; Fugono et al 2001), suggesting that the absorption rate constant of vanadyl species is smaller than the elimination rate constant for these species (Lima & Jusko 1980). Figure 2 summarizes the proposed absorption process of vanadyl species from the gastrointestines after oral administration of ECC.

In conclusion, administration of ECC containing VS was found to improve vanadyl absorption compared with that associated with either GC or the solution. On the basis of the present results, we propose that enteric-coating capsulation of VS is an effective form of administration for enhanced bioavailability of VS.

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