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Improvement of diabetic states in streptozotocin-induced type 1 diabetic rats by vanadyl sulfate in enteric-coated capsules

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Abstract

Chronic oral administration of vanadyl sulfate has recently been shown to improve the state of type 2 diabetic subjects. Mild gastrointestinal symptoms and side effects, however, have been observed in some subjects. To find safer and more effective dosages, we have developed an enteric-coated capsule containing solid vanadyl sulfate (ECC/VS), which enhances the bioavailability of vanadyl sulfate to almost double that of vanadyl sulfate solution. ECC/VS was chronically administered to treat streptozotocin-induced diabetic rats (STZ-rats), an animal model of type 1 diabetes mellitus, and an equivalent blood-glucose-lowering effect was observed at half the doses of vanadyl sulfate alone. In addition, we observed almost the same total vanadium levels in the serum after chronic administration of ECC/VS as those of vanadyl sulfate alone, suggesting that plasma vanadium levels correlate with the hypoglycaemic activity of vanadyl sulfate. These results indicate that oral ECC/VS improves the diabetic state by enhancing the uptake of vanadium in STZ-rats. These findings will be useful in designing clinical trials of vanadyl sulfate for diabetic subjects.

Introduction

In recent years, several metal complexes, such as zinc-containing polaprezinc (Matsukura & Tanaka 2000) and aluminum-containing sucralfate (Lazzaroni et al 1999) as anti-gastric-ulcer drugs, gold-containing auranofin (Simon 2000) as an anti-rheumatoid-arthritis drug and platinum-containing cisplatin (Takahashi et al 2002) as an anticancer drug, have been used as therapeutic agents (Blower 2003). Vanadium ions have been found to exhibit an insulinomimetic action (Shechter & Shisheva 1993; Sakurai et al 2002, 2003; Thompson & Orvig 2004). Chronic oral administration of vanadyl sulfate at a dose of $150\,mg\,kg^{-1}$ daily for 6 weeks was used in clinical trials to treat type 2 diabetic subjects and resulted in improvements in glycaemia and insulin sensitivity, with plasma glucose, fructosamine and haemoglobin A_{1c} (HbA_{1c}) levels being monitored (Cusi et al 2001). An improvement in oral glucose tolerance tests (OGTT) was also observed. Interestingly, the plasma vanadium level in the fasted state was under the detection limit before treatment, but increased to $104 \pm 18\,\mu g\,L^{-1}$ after 6 weeks of oral administration of vanadyl sulfate, as determined by atomic absorption spectrometry. These findings indicate that vanadium levels in the plasma correlate well with the hypoglycaemic activity of vanadyl sulfate, and thus monitoring of the plasma vanadium level is indispensable to determining the effectiveness of administered vanadyl sulfate. However, the authors observed mild gastrointestinal symptoms and side effects, such as diarrhoea and abdominal discomfort, in some subjects during the vanadyl sulfate treatment. Thus, safer and more effective therapeutic methods must be developed.

We have reported that approximately 4.8% of vanadyl species are absorbed following oral administration of vanadyl sulfate, while the bioavailability of vanadyl species is doubled (10.1%) by direct intrajejunal administration of vanadyl sulfate in healthy rats, as estimated by electron spin resonance spectrometry (ESR) (Fugono et al 2001). ESR is useful for detecting paramagnetic vanadyl species after the administration of vanadyl sulfate, which is the active chemical form showing hypoglycaemic effects (Sakurai et al

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1980; Nakai et al 1995). These results suggest that the enteric-coated capsulation of vanadyl sulfate would enhance the bioavailability of vanadyl species. Such observations prompted us to investigate the absorption processes after oral administration of an enteric-coated capsule (ECC) containing solid vanadyl sulfate (ECC/VS) in healthy rats, and we found that the bioavailability of ECC/VS was enhanced approximately twice (9.8%) as much as that of vanadyl sulfate given either as an uncoated gelatin capsule (4.0%) or in saline solution (4.8%) (Fugono et al 2002).

On the basis of our results, we planned to further examine the effectiveness of orally administered ECC/VS in streptozotocin-induced type 1 diabetic rats (STZ-rats). The aim of this study was to compare the hypoglycaemic effects of two different vanadyl sulfate formulations, ECC/VS and vanadyl sulfate saline solution, in STZ-rats, and in turn, to evaluate whether the hyperglycaemia of STZ-rats was equivalently improved by the chronic administration of ECC/VS at half the therapeutic doses of the vanadyl sulfate solution. The improvement of the diabetic state in STZ-rats was demonstrated by the daily blood glucose level and oral glucose tolerance test (OGTT), as well as serum parameters, such as HbA_{1c}, insulin and serum urea nitrogen (BUN) levels, before and after the treatment.

Materials and Methods

Materials

Mini gelatin capsules (diameter 1.5 mm, length 8.0 mm) 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 ($VOSO_4$ 2.3 H_2O) and standard solutions of vanadium (NH_4VO_3 for the atomic absorption spectrometry) were purchased from Wako Pure Chemicals Co. (Osaka, Japan). Streptozotocin (STZ) was purchased from Sigma Chemicals (St Louis, MO). All other compounds used were of analytical reagent grade.

Enteric-coated capsules were prepared by a previously reported method (Fugono et al 2002). Briefly, mini gelatin capsules were filled with solid vanadyl sulfate at a half dose of 5 mg (98 μmol) vanadium per kg of body weight as solution administration. HPMCP solution (40 g L^{-1}) was prepared in a mixture of methylene chloride and methanol (4:1, v/v). After vanadyl-sulfate-containing mini gelatin capsules were placed in the mixture, the solvent was evaporated overnight at 4°C in a refrigerator. The prepared enteric-coated capsules containing solid vanadyl sulfate (ECC/VS) were kept in a desiccator on silica gel until use.

In-vitro dissolution test of ECC/VS

The in-vitro dissolution studies of ECC/VS were carried out at 37°C with stirring on a reduced scale. A test capsule was placed directly into a 10-mL glass vial in which 5 mL of either JPXIV 1st fluid (pH 1.2) or JPXIV 2nd fluid (pH 6.8) was introduced. At 0, 10, 20, 30, 40, 50, 60 and 120 min, 50-μL samples of the dissolution medium were withdrawn and replaced with an equal volume of fresh fluid maintained at 37°C. The test samples were filtered (0.22 μm GV-type filters; Millipore, Molsheim, France) and the dissolved vanadyl sulfate was measured by the ESR method. ESR spectra were measured by an X-band ESR spectrometer (RE-1X; JEOL, Tokyo, Japan). Vanadium levels were determined by the calibration curves obtained from vanadyl species in each JPXIV fluid containing various amounts of vanadyl sulfate. A linear relationship between ESR signal intensities and four different concentrations was found within the range 0.5–5 mmol L^{-1} of vanadyl sulfate, where the correlation coefficient with linear regression was greater than 0.999 for four concentrations in three repeated measurements.

Animals

Male Wistar rats, 7 weeks old, 200–220 g (Shimizu Experimental Material Co., Kyoto, Japan), were housed in a temperature-controlled room at 23 ± 1°C, fed a standard laboratory diet and given free access to water. They were fasted overnight for 12 h and given STZ (35 mg kg^{-1}) in 0.1 mol L^{-1} citrate buffer (pH 5.0) by a single intravenous injection to induce type 1 diabetes mellitus (STZ-rats). During the experiments, blood samples for monitoring glucose levels were obtained from the tail vein of the rats, and blood glucose levels were measured daily using the Fuji Dri-Chem Slide and Fuji Dri-Chem 1000 (Fuji Film Med Co., Tokyo, Japan). While the healthy Wistar rats expressed the normal ranges of both blood glucose (5–8 mM; 90–140 mg dL^{-1}) and serum insulin levels (50–80 mU L^{-1}), STZ-rats showing blood glucose levels higher than 16 mM (288 mg dL^{-1}) and serum insulin levels lower than 10 mU L^{-1} were used as type 1 model diabetic animals for chronic administration of vanadyl sulfate in each form in this study. All experiments were approved by the Experimental Animal Research Committee of Kyoto Pharmaceutical University (KPU), and were performed according to the Guidelines for Animal Experimentation of KPU.

Chronic oral administration of ECC/VS, vanadyl sulfate saline solution or saline in STZ-rats

One enteric-coated capsule containing solid vanadyl sulfate at a dose of 5 mg (98 μmol) vanadium per kg of body weight was orally administered to STZ-rats daily for 21 day 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). Vanadyl sulfate saline solution at a dose of 10 mg (196 μmol) vanadium per kg of body weight, or the vehicle saline, was administered at the same volume of 0.5 mL.

Measurement of serum biochemical parameters and total vanadium levels in the serum

Serum parameters and total vanadium level were determined before and after chronic administration of the compounds for 21 days. For the serum collection, STZ-rats were

fasted overnight for 12 h. Serum glucose levels (SGLU) and BUN were determined with a Fuji Dri-Chem 3000 (Fuji Film Med Co., Tokyo, Japan). Serum insulin was measured by a GLAZYME insulin-EIA test (Wako Pure Chemicals, Osaka, Japan) and blood HbA_{1c} was measured by a DCA 2000 system (Bayer Co., Tokyo, Japan). Total vanadium levels in the serum were determined by ESR after complete reduction of the digested samples by ascorbic acid. In brief, the serum samples digested with 60% HNO_3, 30% H_2O_2, and 60% $HClO_4$ were reduced by ascorbic acid, with 10 μL of ascorbic acid in 0.1 mol L^{-1} being added to 90 μL of the digested sample, and ESR spectra were measured by an X-band ESR spectrometer (RE-1X, JEOL, Tokyo, Japan). Total vanadium levels were thus determined by the calibration curve obtained from vanadyl species in the solution containing various amounts of ammonium vanadate in addition to ascorbic acid, as for the measurement of serum samples. A linear relationship between ESR signal intensities and four different concentrations was found within the range of 5–40 μmol L^{-1} of vanadium, where the correlation coefficient with linear regression was greater than 0.999 for four concentrations in three repeated measurements. The detection limit of total vanadium was 2 μmol L^{-1} at $S/N = 2$.

Oral glucose tolerance test

An oral glucose tolerance test (OGTT) was performed to appraise whether the diabetes mellitus was improved after ECC/VS administration. After chronic administration of vanadyl sulfate in each form for 21 days, 40% glucose solution (2 g kg^{-1}) was orally administered to STZ-rats under the fasted condition for 12 h. Blood samples were periodically obtained from the tail vein of the rats at 0, 15, 30, 60, 90, 120, 180 and 240 min after the administration of glucose solution, and blood glucose levels were determined. Glucose tolerance ability was evaluated on the basis of the area under the time–blood glucose level curve (AUC) calculated by the trapezoidal method (Yamaoka et al 1978).

Statistical analysis

All experimental results are expressed as the arithmetic mean ± standard deviation for 4 rats. The statistical analysis was performed using the Mann–Whitney U test or the Kruskal–Wallis test followed by a post-hoc test of Dunn's multiple comparison test at 5% ($P < 0.05$) or 1% ($P < 0.01$) significance levels.

Results

To evaluate the effect of an enteric-coated preparation on the dissolution of the capsule, ECC/VS were assessed by in-vitro release studies. Compared with the conventional commercial gelatin capsule from which 95% vanadyl sulfate was released within 30 min in both JPXIV 1st (pH 1.2) and 2nd (pH 6.8) fluids, a remarkable improvement of the in-vitro vanadyl sulfate dissolution was achieved by the enteric-coated capsules from which no vanadyl sulfate was released within 120 min in JPXIV 1st fluid but was gradually released from 10 min and reached the 95% level at 60 min in JPXIV 2nd fluid, indicating that vanadyl sulfate is not released from enteric-coated capsules in the stomach but in the lower intestine such as the jejunum or ileum. In our previous investigation (Fugono et al 2002), we showed that the bioavailability of vanadyl sulfate from enteric-coated capsules (9.8%) was almost double that from either gelatin capsules (4.0%) or the solution (4.8%). The enhanced bioavailability of vanadyl sulfate was due to the delivery and disintegration of enteric-coated capsules at the ileum where vanadyl sulfate was absorbed more thoroughly than at other gastrointestinal sites (Fugono et al 2001).

Changes in blood glucose levels in STZ-rats that were administered two vanadyl sulfate formulations, one in saline solution (10 mg vanadium per kg of body weight) and the other as ECC/VS (5 mg vanadium per kg of body weight), for 21 days are shown in Figure 1. High blood glucose levels in STZ-rats were gradually reduced for 21 days with chronic oral administration of both formulations, but normal blood glucose levels (90–140 mg dL^{-1}) were not maintained on average. In contrast, the blood glucose levels of STZ-rats treated with saline (control group) gradually increased to almost 600 mg dL^{-1}. These results indicate that an equivalent blood-glucose-lowering effect was achieved using the ECC/VS at doses half those of vanadyl sulfate alone. We then performed OGTT to evaluate whether the diabetes states of STZ-rats given ECC/VS and vanadyl sulfate alone were actually improved. Blood glucose levels in STZ-rats treated with vanadyl sulfate in each formulation were found to be significantly lower than those of the control at each sampling time after 1 h of OGT (Figure 2). The areas under time–blood glucose level

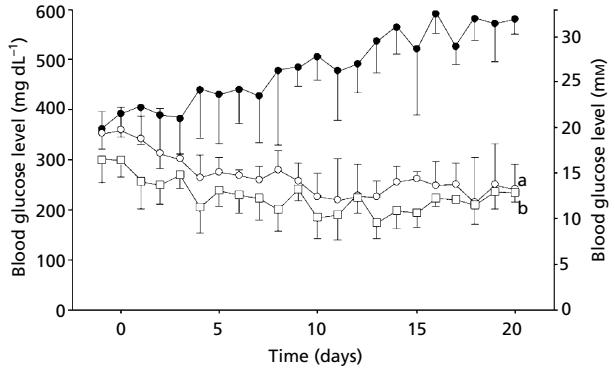

Figure 1 Changes in blood glucose levels after chronic oral administration of vanadyl sulfate in the form of a solution (○) or enteric-coated capsules (□) or saline (●) to STZ-rats for 21 days. Rats were given vanadyl sulfate at doses of 10 mg (solution) and 5 mg (enteric-coated capsules) vanadium per kg of body weight. Each symbol represents the mean ± s.d., n = 4. [a] $P < 0.05$, the 0th vs 20th days of solution; [b] $P < 0.05$, the 0th vs 20th days of enteric-coated capsules, using the Mann–Whitney U test.

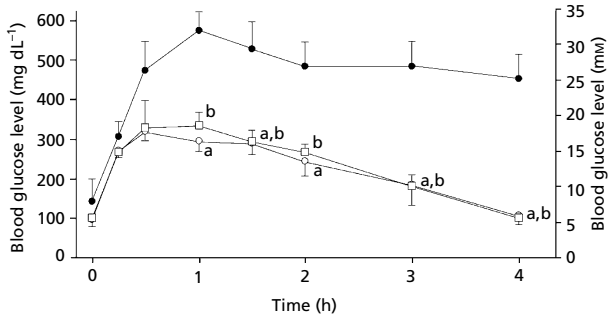

Figure 2 Changes in blood glucose levels after oral glucose loading ($2\,g\,kg^{-1}$) to STZ-rats treated with vanadyl sulfate in the form of a solution (○) or enteric-coated capsules (□) or saline (●) for 21 days. Rats were given vanadyl sulfate in the same manner as described in Figure 1. Each symbol represents the mean ± s.d., n = 4. [a]$P < 0.05$ saline vs solution; [b]$P < 0.05$ saline vs enteric-coated capsules, using the Kruskal–Wallis test followed by a post-hoc test of Dunn's multiple comparison test.

curves (AUCs) after glucose loading in STZ-rats treated with vanadyl sulfate in enteric-coated capsule ($950 \pm 70\,mg\,dL^{-1}\,h$) or solution ($920 \pm 90\,mg\,dL^{-1}\,h$) formulations were significantly smaller than those of the control ($1890 \pm 200\,mg\,dL^{-1}\,h$) at 5% ($P < 0.05$) levels.

The serum parameters and total vanadium levels in the serum of STZ-rats treated with both formulations are summarized in Table 1. SGLU and HbA_{1c} levels of STZ-rats treated with vanadyl sulfate in each formulation were significantly lower than those of the control rats, respectively. BUN levels also significantly decreased with the administration of each formulation. In addition, although total vanadium levels were lower than the detection limit before administration of vanadyl sulfate in either form, they significantly increased to almost the same levels in the serum after oral administration of each formulation for 21 days.

Discussion

Since it was found that the vanadate ion (+5 oxidation state of vanadium), given orally in drinking water, produces a blood-glucose-lowering effect (Meyerovitch et al 1987), we have attempted to develop new vanadyl complexes that are relatively less toxic and that have a more active chemical form for developing insulinomimetic activity (Sakurai et al 2002, 2003). To achieve a hypoglycaemic effect by a safer administration regimen at lower doses, we first investigated the pharmacokinetic characteristics of vanadyl sulfate in terms of gastrointestinal absorption, and found that the vanadyl species is absorbed more thoroughly at the ileum than at other gastrointestinal sites in healthy rats given vanadyl sulfate (Fugono et al 2001). As such, we developed an enteric-coated capsule that is delivered to the intestine and is disintegrated there to enhance the bioavailability of vanadyl sulfate after oral administration, and we confirmed that the bioavailability of ECC/VS was double that of vanadyl sulfate alone (Fugono et al 2002).

According to a WHO report (WHO 1985), multifactorial diabetes mellitus is mainly classified as either insulin-dependent type 1 diabetes mellitus caused by destruction of pancreas β-cells or non-insulin-dependent type 2 diabetes mellitus caused by factors such as ageing, obesity, stress or other environmental factors. First, we then continued our investigation to compare the hypoglycaemic effects of two vanadyl sulfate formulations, ECC/VS and vanadyl sulfate saline solution, in STZ-induced type 1 diabetes mellitus model rats (Figure 1). Hyperglycaemia of STZ-rats was significantly improved by the chronic oral administration of both vanadyl sulfate formulations, and the half doses of vanadyl sulfate in enteric-coated capsules were found to achieve a therapeutic effect equivalent to that after administering vanadyl sulfate alone. The improvement of the diabetic state in STZ-rats was demonstrated by OGTT (Figure 2) as well as the serum parameters (Table 1). In addition, although diarrhoea was continuously observed in STZ-rats treated with vanadyl sulfate saline solution at a daily dose of 10 mg vanadium per kg of body weight during the treatment period, little diarrhoea was observed in STZ-rats treated with ECC/VS at a daily dose of 5 mg vanadium per kg of body weight, indicating that the achievement of lowering the therapeutic dose leads to suppression of the gastrointestinal side effects during the vanadyl sulfate treatment.

HbA_{1c} levels, which indicate the average blood glucose levels over 1 month (Kitazawa et al 1996), in STZ-rats

Table 1 Serum and blood parameters in STZ-rats treated with vanadyl sulfate in solution and enteric-coated capsule formulations

	Administration method	Dose ($mg\,vanadium\,kg^{-1}$)	SGLU ($mg\,dL^{-1}$)	Insulin ($mU\,L^{-1}$)	HbA_{1c} (%)	BUN ($mg\,dL^{-1}$)	Total serum vanadium ($\mu mol\,L^{-1}$)
0 day	No treatment		206 ± 14	4.9 ± 3.0	4.5 ± 0.8	20.0 ± 3.7	n.d.
21 day	Saline (control)		333 ± 41	6.1 ± 2.0	7.0 ± 0.7	34.6 ± 1.1	n.d.
	Solution	10	215 ± 18*	5.1 ± 3.1	4.6 ± 0.3*	14.5 ± 1.5*	8.9 ± 4.9
	Enteric-coated capsules	5	197 ± 23*	2.7 ± 1.6	4.3 ± 0.4*	17.6 ± 5.8*	8.0 ± 0.5

SGLU, serum glucose level; insulin, serum insulin level; HbA_{1c}, blood haemoglobin A_{1c} level; BUN, serum urea nitrogen; n.d., not detected. Each value is represented as the mean ± s.d., n = 4. *$P < 0.05$ vs control, using the Kruskal–Wallis test followed by a post-hoc test of Dunn's multiple comparison test.

treated with each vanadyl sulfate formulation significantly decreased in comparison with those of the control rats treated with saline. HbA_{1c} levels are known to increase with the maintenance of diabetic states. In fact, the HbA_{1c} levels of the control rats increased after 21 days, while those of STZ-rats after treatments with vanadyl sulfate in both formulations decreased compared with levels before the treatments, suggesting that treatment with vanadyl sulfate suppresses the seriousness of diabetic states. Although the insulin levels of STZ-rats treated with ECC/VS were not significantly different from those of the control rats treated with saline, the blood glucose levels were lowered, indicating that the insulinomimetic effect of vanadyl sulfate was not peripheral, as proposed previously (Sakurai et al 1990). The decrease in BUN levels in STZ-rats treated with both formulations suggests that vanadyl sulfate given at such doses and in such dosage forms is less toxic to the renal function. Total vanadium levels in the serum of STZ-rats after treatment with vanadyl sulfate in each formulation for 21 days increased compared with those of control rats treated with saline. Almost the same total vanadium levels were found in rats treated with both vanadyl sulfate formulations, even though the doses of the two formulations were different, with that of ECC/VS being half that of vanadyl sulfate solution. These findings indicate that vanadium levels in the blood are an important factor in lowering blood glucose levels in STZ-rats, as suggested in the human trial (Cusi et al 2001). At 7 weeks after discontinuation of the vanadyl sulfate treatment in STZ-rats used here, total vanadium levels in the serum of rats dropped again to an undetectable range below $2\,\mu mol\,L^{-1}$, and SGLU levels increased over $300\,mg\,dL^{-1}$.

In conclusion, the results of our pharmacokinetic study of vanadyl sulfate in the blood of normal rats indicate that oral administration of ECC/VS lowers blood glucose levels in STZ-rats, giving an equivalent hypoglycaemic effect at half the dose of vanadyl sulfate alone, as supported by vanadium levels in the blood. These findings should be useful in designing clinical trials of vanadyl sulfate for diabetic subjects (Jones 2004).

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