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Oral therapy of L-glutamic acid γ-monohydroxamate-vanadium (2:1) complex: Improvement of blood glucose profile in different types of diabetic rodents*

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Abstract: We report that oral administration of vanadium (+5) combined with L-glutamic acid γ-monohydroxamate at 1:2 stoichiometry [L-Glu(γ)HXM•VO_3^-] is highly effective in reducing blood glucose levels (BGLs) in a wide variety of diabetic rodents. In streptozocin-treated rats, a single administration (0.28 mmol/kg body wt) decreased BGL from 490 to 360 mg/dl within 1 h of administration, keeping this reduced level for additional 22 h, and a daily dose of 0.14 mmol/kg was found optimal. In Zucker diabetic fatty (ZDF) rats, a single dose of 0.14 mmol/kg normalized BGL within 8 h of administration, and maintained normal value for additional two days. In db/db mice, a single L-Glu(γ)HXM•VO_3^- administration of 0.2 mmol/kg decreased BGL from 500 ± 50 to 240 ± 20 mg/dl at 2 h, but was less effective afterwards. In high-carbohydrate (CHO)-fed *Psammomis obesus*, a single oral dose (0.14 mmol/kg) normalized BGL over a period of two days, and a daily dose of 0.07 mmol/kg/d, at the time *P. obesus* was transferred from low- to high-CHO diet, fully arrested the development of hyperglycemia characterizing this diabetic rodent. Finally, we found that the index of toxicity of orally administered L-GLU(γ)HXM-vanadate in rodents is 5–7 times lower than that of free sodium vanadate.

Keywords: Diabetes; vanadium; insulinomimetic agents; alternative pathways; diabetic rodents; antidiabetic agents.

INTRODUCTION

Vanadium salts mimic most of the rapid metabolic effects of insulin on the main target tissues of the hormone in vitro [1–8]. In addition, vanadium salts induce normoglycemia and improve glucose homeostasis in insulin-deficient [9–14] and -resistant diabetic rodents in vivo [15–18]. Data has been accumulating showing that the metabolic actions of vanadium are mediated through an insulin-receptor tyrosine kinase independent mechanism [5,19–22]. The key events of this system appear to involve inhibition of protein-phosphotyrosine phosphatases and activation of nonreceptor protein tyrosine kinases [23–26]. With regard to glucose metabolism, vanadium acts at a site(s) preceding the activation of phosphatidylinositol-3-kinase, and as an antilipolytic agent, it acts downstream to PI3-kinase activation [27].

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Various organically chelated vanadium compounds are more potent in facilitating insulin-like effects in vitro and in vivo than free vanadium salts [28–30]. Recently, we found that the L-isomer of glutamic-acid γ-monohydroxamate [Glu(γ)HXM] is particularly active: in streptozocin (STZ) rats, intraperitoneal administered L-Glu(γ)HXM·VO_3^- (2:1 stochiometry) potentiated vanadium-induced normalization of circulating glucose levels by 4–7-fold [31]. Moreover, in rat adipocytes in vitro, L-Glu(γ)HXM itself, in the absence of vanadium, activated lipogenesis to 30 ± 5 % of maximal stimulation. It appears that L-Glu(γ)HXM permeates into cells through the glutamine transporter and associates with the minute available quantities (20 nM) of intracellular vanadium, thus turning it into an insulinomimetically active species [31,32].

Preliminary clinical studies have been already performed with low doses of free vanadium to minimize toxicity. Although administered in subeffective doses, several beneficial effects were observed and documented [33–36]. Hence, vanadium might be used in the future care of diabetes in humans, provided that the insulinomimetic efficacy of this metalooxide is significantly elevated, and its index of toxicity is considerably reduced.

In this work, we investigated the glucose-lowering potency of orally administered (gavage) L-Glu(γ)HXM·VO_3^- in a variety of hyperglycemic diabetic rodents following a single administration and daily-administrating protocols. The hyperglycemic state of the various types of rodents stemmed from distinctly different pathological mechanisms (reviewed in ref. [37]). Our specific intention was to determine whether this oral therapy antagonizes hyperglycemia efficiently and, if so, whether it is equipotent to tackle any "type" of hyperglycemia, independent of its etiology. The diabetic rodents studied here included the STZ rat, a widely used model for investigating the deteriorating effects of chronic hyperglycemia, known to be reversible by insulin [38–40] or by vanadium therapy [9–10]; the genetic hyperglycemic, hyperinsulinemic db/db mouse, which does not respond to exogeneously administered insulin [41]; the male ZDF rat whose onset of Type II diabetes mellitus resembles the one developed in human [42]; and the sand rat (*Psammomys obesus*), which develops overt diabetes upon its transfer from low- to high-carbohydrate (CHO) diet [43].

EXPERIMENTAL SUBJECTS

Materials

Sodium metavanadate ($NaVO_3$), streptozocin, and L-Glu(γ)HXM were from Sigma Chemical Co. L-Glu(γ)HXM·VO_3^- (2:1) was prepared by dissolving 5 g of L-Glu(γ)hydroxamate in 150 ml H_2O, and 1.88 g $NaVO_3$ in another sample of 150 ml H_2O. The $NaVO_3$ solution was stirred at 25 °C for 3–4 h until fully dissolved. The two solutions were combined to obtain a brown solution and then lyophilized to yield a gray powder [31,32].

Animals

Streptozocin-treated rats. Male Wistar rats (180–200 g) received an intravenous injection of freshly prepared solution of streptozocin (55 mg/kg body wt) in 0.1 M citrate buffer (pH 4.5, ref. [10]).

db/db Mice (db/+) were purchased from Jackson Laboratory (Bar Harbor, ME, USA) and used at 6–8 weeks of age.

Male Zucker diabetic fatty rats (ZDF/Gmi; TM fa/fa) were purchased from Genetic Models (Indianapolis, IN, USA). Animals were fed *ad libitum* a standard purina chow, and kept under a constant 12 h light/dark cycle.

Psammomys obesus used in this study are a selected diabetes-prone line from an established colony of the Animal Farm at the Hebrew University, Hadassah Medical School, Jerusalem, Israel [44]. Animals were fed either a low-CHO (low-energy) diet supplying digestible energy of 2.4 kcal/g or a high-CHO (high-energy) diet containing 3.1 kcal/g of digestible energy.

Other procedures

Blood glucose was determined by the glucose oxidase method applied to blood samples taken from the tail vein. A glucose analyzer (Beckman Instruments, Fullerton, CA) was used.

Mode of administration. Unlike previous studies where $NaVO_3$ was often supplied in drinking water, a gavage-mode of administration was used here exclusively to ensure quantity control of the dosage. Briefly, prior to administration lyophilized L-Glu(γ)HXM•VO_3^- (2:1) was dissolved in H_2O at a concentration of 0.1 mmol/ml (44.5 mg/ml). The animals were weighed, and the amount to be administered by gavage was calculated accordingly. Ordinarily, a volume of 0.4 to 0.6 ml/rat was administered. Control diabetic rats received the same volume of H_2O.

RESULTS

A single administration of L-Glu(γ)HXM•VO_3^- *facilitates rapid and prolonged glucose-lowering effect in STZ-rats*. As shown in Fig. 1, the high blood glucose levels (BGLs) characterizing the STZ rat model 5 days after the induction of diabetes (490 ± 40 mg/dl) were substantially decreased following a single oral administration of L-Glu(γ)HXM•VO_3^- (2:1) at a dose of 0.28 mmol/kg body wt. Circulating glucose levels fell to 360 ± 20 mg/dl at 1 h after administration, and remained low over a period of 22 h (300 ± 40 mg/dl) before returning to hyperglycemic levels. Thus, a single oral dose of L-Glu(γ)HXM•VO_3^- (2:1) facilitates a rapid and prolonged glucose-lowering pattern in this diabetic rat model.

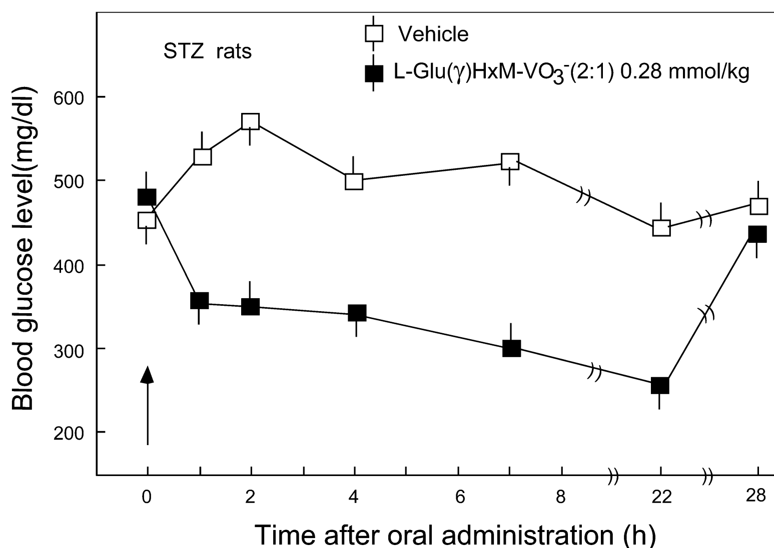

Fig. 1 *Glucose-lowering pattern of L-Glu(γ)HXM•VO_3^- following a single oral administration in STZ rats*. Five days after disease induction, streptozocin-treated rats received orally, by gavage, either vehicle (H_2O, 1.0 ml, □) or L-Glu(γ)HXM•VO_3^- (2:1, ■) at a dose of 25 mg/rat (0.28 mmol/kg). BGLs were monitored at the indicated time points. Each point is the arithmetic mean of plasma glucose of 6 STZ rats ± SEM.

A daily administrating protocol of L-Glu(γ)HXM•VO_3^- (2:1) in STZ rats. In Fig. 2, STZ rats received a daily dose of L-Glu(γ)HXM•VO_3^- (2:1) over a period of 7 days (0.14 mmol/kg/d) and BGL was monitored during and following the completion of this therapy. Circulating glucose levels largely decreased (from 427 ± 20 to 190 ± 15 mg/dl) on the third day of this therapy and remained low as long as L-Glu(γ)HXM•VO_3^- (2:1) was orally administered. Following termination of this therapy, BGLs were gradually elevated, but remained considerably lower compared to vehicle-treated STZ rats

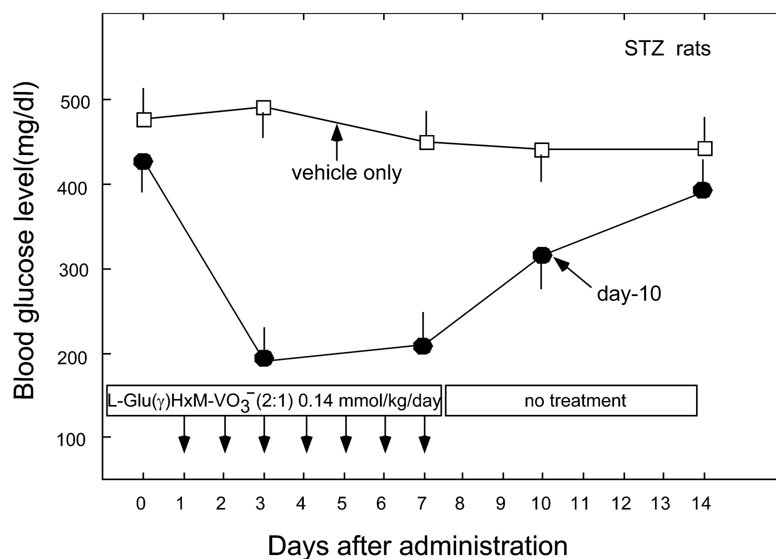

Fig. 2 *Glucose-lowering pattern of* L*-Glu(γ)HXM•VO_3^- (2:1) in STZ rats following daily administration.* STZ rats, 5 days after the induction of the disease, received by gavage either vehicle (H_2O, 1.0 ml, □) or L-Glu(γ)HXM•VO_3^- (2:1, ■) at a dose of 0.14 mmol/kg/d over a period of 7 days. BGLs were monitored at the indicated time points. Each group consistsed of 6 STZ rats. Values are means ± SEM.

(Fig. 1). Returning to hyperglycemic levels proceeded with a $t_{1/2}$ value of about 3 days (day 10 in the figure) after cessation of therapy.

Glucose-lowering pattern of L*-Glu(γ)HXM•VO_3^- (2:1) following a single administration in db/db mice*. The db/db mouse is a Type II genetic diabetic model in which a marked insulin resistance leads to overt diabetes despite very high plasma insulin levels [41]. A single oral administration of L-Glu(γ)HXM•VO_3^- (2:1) at a dose of 0.22 mmol/kg lowered BGLs within 2 h of administration by 52 % (from 500 ± 50 to 240 ± 20 mg/dl (Fig. 3). Circulating BGLs were elevated subsequently, but still remained low by 15–25 % at 24 and 48 h after administration relative to vehicle-treated db/db mice (Fig. 3). In this set of experiments, the glucose-lowering potency of singly administered troglitazone, at a dose of 0.07 mmol/kg body wt, was investigated as well. Troglitazone reduces BGLs in a variety of insulin-resistant rodents, including db/db mice, presumably by increasing the insulin sensitivity of peripheral tissues [45]. As shown in Fig. 3, L-Glu(γ)HXM•VO_3^- was considerably superior to troglitazone in decreasing BGLs in db/db mice.

Studies in ZDF-rats: L*-Glu(γ)HXM•VO_3^- normalizes circulating glucose levels in this rat model*. Homozygous ZDF male rats develop progressive resistance to insulin and glucose intolerance at 3–8 weeks of age, and then become overtly diabetic (hypergycemic and hyperinsulinemic) at 8–10 weeks of age. Beyond week 10, this diabetic rodent becomes hypoinsulinemic as well due to irreversible damage to pancreatic β-cells that takes place following a prolonged state of hyperglycemia [46].

In Fig. 4A, 10-week-old ZDF male rats having BGL of 470 ± 30 mg/dl were orally administered increasing dosage of L-Glu(γ)HXM•VO_3^-. Circulating glucose levels were monitored 24 h after administration. BGLs decreased as a function of the dosage applied, being significantly reduced at a dose of 0.07 mmol/kg (decreased to 380 ± 20 mg/dl), nearly normalized at a dose of 0.14 mmol/kg, and reaching mild hypoglycemic value (60 ± 10 mg/dl) at a dose of 0.28 mmol/kg body wt (Fig. 4A).

Figure 4B shows the time-dependent glucose lowering profile following a single oral administration of L-Glu(γ)HXM•VO_3^- (2:1) at a dose of 0.14 mmol/kg body wt. As shown, BGL dropped significantly already 1 h after administration and continued to decrease over a period of 8 h (110 ± 10 mg/dl,

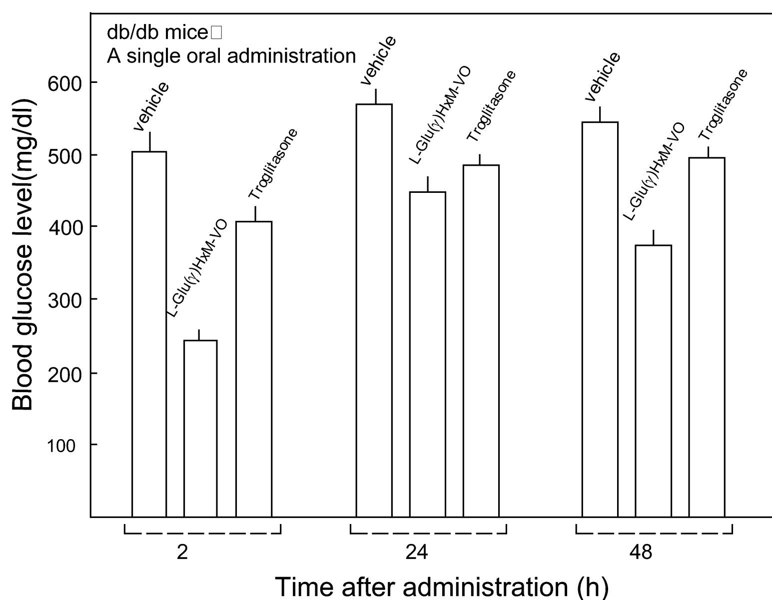

Fig. 3 *Effect of L-Glu(γ)HXM·VO_3^- (2:1) and of troglitazone on BGLs in db/db mice*. db/db Mice received a single oral dose by gavage of either vehicle (1.0 ml, H_2O), L-Glu(γ)HXM·VO_3^- (2:1, 0.22 mmol/kg), or troglitazone (0.07 mmol/kg). BGLs were determined at 2, 24, and 48 h after administration. Each group consisted of 6 db/db mice. Values are means ± SEM.

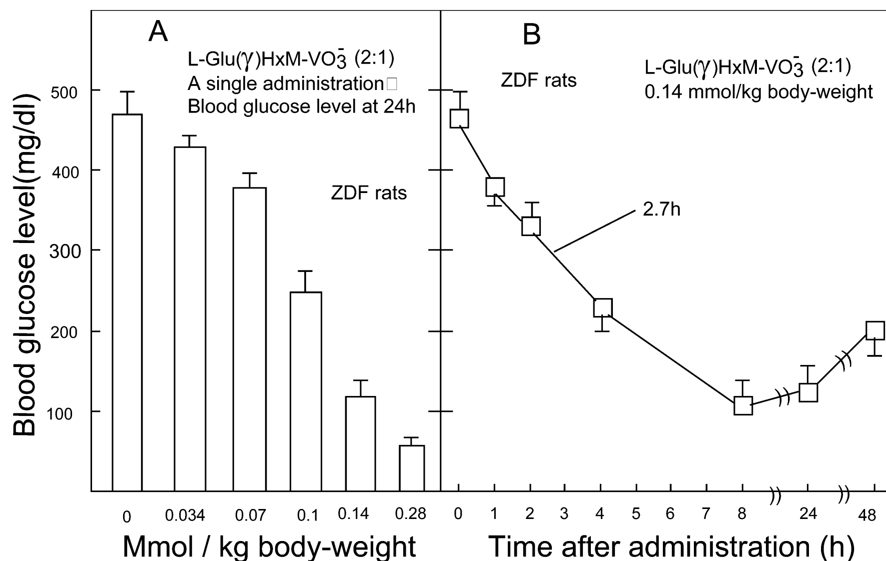

Fig. 4 *Effect of a single oral administration of L-Glu(γ)HXM·VO_3^- (2:1) on BGLs of ZDF rats*. (A) 10-week-old ZDF male rats (n = 6 for each group) received orally by gavage either vehicle (1.0 ml H_2O) or the indicated doses of L-Glu(γ)HXM·VO_3^- (2:1*)*. Circulating glucose levels were determined 24 h after administration (B) A group of ZDF male rats (n = 6) received orally by gavage L-Glu(γ)HXM·VO_3^- (2:1) at a dose of 0.14 mmol/kg. Circulating glucose levels were monitored at the time points indicated. Values are means ± SEM.

$t_{1/2}$ = 2.7 h). A stable state of normoglycemia was then retained for a period of over 24 h after administration (Fig. 4B).

Daily administration of L*-Glu(γ)HXM•*VO_3^- *in ZDF male rats*. In the experiments summarized in Fig. 5, 12-week-old ZDF male rats (n = 6 in each group) received either vehicle or L-Glu(γ)HXM•VO_3^- at a dose of 0.07 mmol/kg/d. Following 20 days of this therapy, circulating glucose levels were monitored. As shown in Fig. 5, BGLs in the L-Glu(γ)HXM•VO_3^- treated group were lowered by about 20 % (360 ± 100 mg/dl) relative to vehicle-treated ZDF male rats. Circulating glucose levels remained lower by 25 ± 7 % as long as L-Glu(γ)HXM•VO_3^- was administered. Thus, this therapy lowers BGLs to some extent, but is considerably less effective in 12-week-old ZDF male rats.

Studies in P. obesus*:* L*-Glu(γ)HXM•*VO_3^- *normalizes circulating glucose levels. P. obesus* is unique in being nondiabetic as long as it is maintained on low-CHO diet, but it lapses into severe diabetes when overfed [44]. This model is therefore reminiscent of the deteriorating effects of overfeeding in human diabetic patients and the appearance of the disease in populations that were traditionally fed a low-energy diet. This animal model may also assist in investigating the progressive increase in the incidence of NIDDM patients in Western countries (reviewed in ref. [44]). From an experimental point of view, the selected diabetes-prone line of *P. obesus* is classified into several states: those sand rats that are maintained on low-CHO diet and are therefore normoglycemic and normoinsulinemic (state A); those placed on high-CHO diet over a period of 2–3 weeks and are hyperglycemic and hyperinsulinemic (state C); and those maintained on high-CHO diet for 5–8 weeks (state D). The latter are both hyperglycemic and hypoinsulinemic as a result of apoptotic collapse of pancreatic β-cells that takes place after a prolonged period of hyperglycemia and oversecretion of insulin [47].

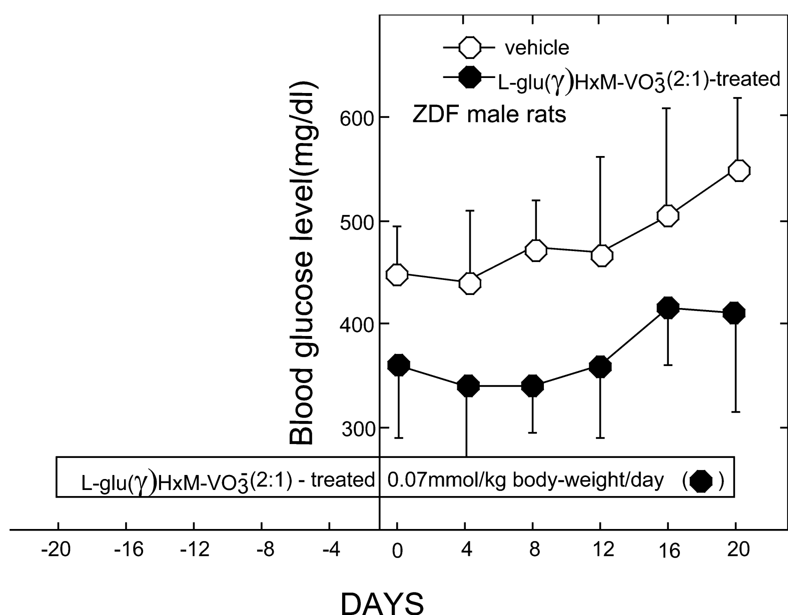

Fig. 5 *Daily administration of* L*-Glu(γ)HXM•*VO_3^- *(2:1) in ZDF rats*. 12-week-old ZDF male rats (n = 6 in each group) received daily by gavage either vehicle (H_2O, O) or L-Glu(γ)HXM•VO_3^- (2:1 ●) at a dose of 0.07 mmol/kg/d. After 20 days, circulating glucose levels were monitored at the time points indicated in the figure. Values are means ± SEM.

Figure 6A shows the L-Glu(γ)HXM•VO_3^- (2:1) dose-dependent decrease in BGL in state-C *P. obesus*. Circulating BGLs were determined 24 h after a single oral administration of the doses indicated in the figure. BGL values were slightly decreased at a dose of 0.07 mmol/kg body wt (from 310 ± 10

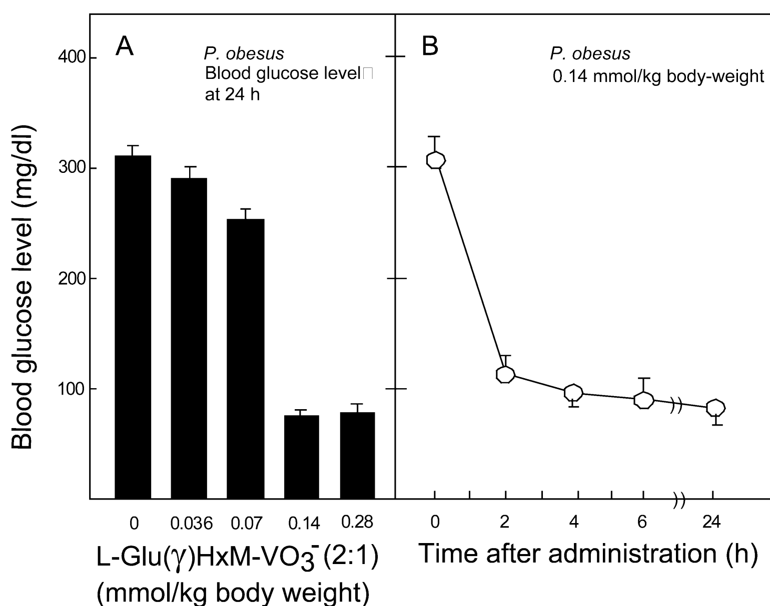

Fig. 6 *Effect of a single administration of* L*-Glu(γ)HXM·*VO_3^- *(2:1) on BGLs in* P. obesus. (A) Groups of *P. obesus* (*n* = 4 for each group), placed on high-CHO diet for 2–3 weeks (state C), received orally by gavage L-Glu(γ)HXM·VO_3^- (2:1) at doses indicated in the figure. BGLs were monitored 24 h after administrations. (B) A group of *P. obesus* (state C, *n* = 4) received orally by gavage L-Glu(γ)HXM·VO_3^- (2:1) at a dose of 0.14 mmol/kg. BGLs were determined at the time points indicated in the figure. Values are means ± SEM.

to 255 ± 15 mg/dl) and fully normalized following administration of 0.14 mmol/kg (80 ± 7 mg/dl). A higher dose of L-Glu(γ)HXM·VO_3^- (0.28 mmol/kg) did not facilitate further glucose-lowering effect, nor hypoglycemia (70 ± 7 mg/dl, Fig. 6A).

As shown in Fig. 6B, the glucose-lowering effect of L-Glu(γ)HXM·VO_3^- following a single administration (0.14 mmol/kg) was nearly complete 2 h after administration (115 ± 10 mg/dl). Stable normoglycemic level was then maintained over a period of 24 h (Fig. 6B).

L-Glu(γ)HXM·VO_3^- *therapy arrests the onset of hyperglycemia in* P. obesus *placed on high-CHO diet*. In the experiment summarized in Fig. 7, *P. obesus* were normoglycemic after being initially kept on low-CHO diet for a period of 8 days (days –2 and –1 in the figure). A group of *P. obesus* (*n* = 10) were then placed on a daily oral L-Glu(γ)HXM·VO_3^- therapy (0.07 mmol/kg/d) over a period of 7 days, starting 1 day before being transferred from low- to high-CHO diet (day –1 in the figure). A control group (*n* = 10) receiving vehicle only was also placed on high-CHO diet. In the L-Glu(γ)HXM·VO_3^- untreated group BGLs were progressively increased, reaching hyperglycemic values of above 275 ± 90 mg/dl 6 days after the onset of the high-CHO diet. L-Glu(γ)HXM·VO_3^- therapy fully arrested high-CHO-induced hyperglycemia in these rodents. Circulating glucose levels were normoglycemic during the treatment (70 ± 10 mg/dl) and remained low for an additional 5 days after cessation of therapy. Both L-Glu(γ)HXM·VO_3^--treated and untreated groups gained weight at a nearly similar rate. Mean daily food intake during the course of L-Glu(γ)HXM·VO_3^- therapy was reduced by less than 7 % in comparison to the untreated group (not shown).

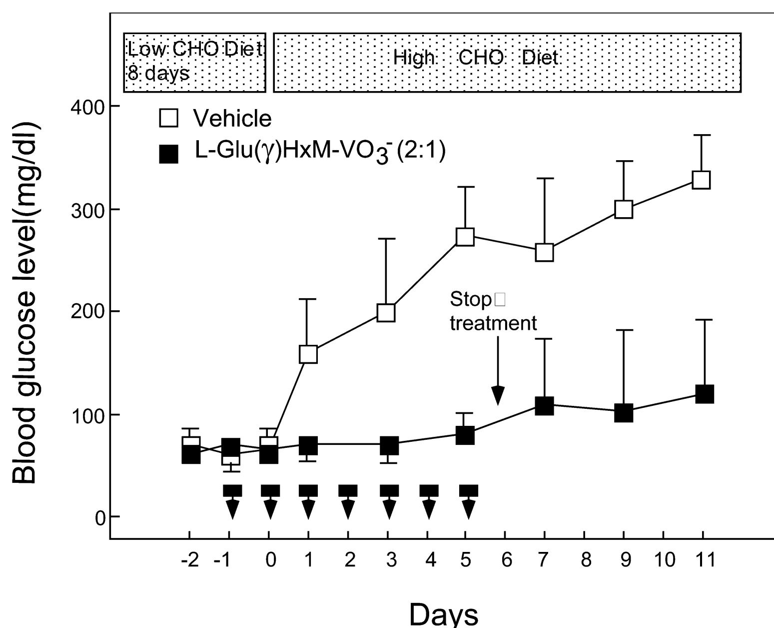

Fig. 7 *L-Glu(γ)HXM·VO_3^- (2:1) therapy arrests the onset of hyperglycemia in high-CHO* P. obesus. After 8 days on low-CHO diet, sand rat*s* were divided into 2 groups (n = 10 per group) which received orally by gavage either vehicle (1.0 ml H_2O, □), or L-Glu(γ)HXM·VO_3^- at a dose of 0.07 mmol/kg/d over a period of 7 days (starting from day –1 in the figure). Both groups were then transferred to high-CHO diet (starting at day 0 in the figure). BGLs were monitored at the time points indicated in the figure. Each point represents the arithmetic mean of plasma glucose ± SEM.

Lower index of toxicity. The three studies summarized in Table 1 were carried out to assess whether index of toxicity of L-Glu(γ)HXM·VO_3^- (2:1) was significantly lower in comparison to that of free sodium metavanadate ($NaVO_3$). In the first set of experiments, CD_1 mice received by gavage daily dose up to 143 µmoles/kg/d of either sodium metavanadate ($NaVO_3$) or equimolar concentrations of L-Glu(γ)HXM·VO_3^- (2:1) over a period of 1 week (n = 8 for each group). In the $NaVO_3$-treated group, 2 mice died on day 3 of the treatment, whereas none in the L-Glu(γ)HXM·VO_3^--treated group. In the second set of experiments, Wistar male rats (200 ± 10 g) received by oral gavage increasing doses of L-Glu(γ)HXM·VO_3^- (2:1) over a period of 28 days. Daily administration of up to 0.3 mmol/kg/d was found appropriate, and all rats remained alive. Treatment did not cause a rise in any of the serum components analyzed, including urea, creatinine, glutamic-oxaloacetic acid transaminase, bilirubin, or alkaline phosphatase, indicating no impairment in renal or liver functions (summarized in Table 1). Also, no alterations in heart, liver, and kidney tissues could be detected by histological examinations (not shown).

Oral absorption of vanadium salts is highly variable and might be strongly affected by dietary components [48]. In the last set of experiments, we therefore determined the lethal-dose$_{50}$ (LD_{50}) for $NaVO_3$ and for L-Glu(γ)HXM·VO_3^- (2:1) in Wistar rats by a single intraperitoneal administration. These values were found 0.1 ± 0.02 and 0.23 ± 0.04 mmol/kg for $NaVO_3$ and for L-Glu(γ)HXM·VO_3^- (2:1), respectively. Thus, in the case of intraperitoneal administrations in rats, LD_{50} values are reduced by a factor of 2.3 compared to that of sodium metavanadate.

Table 1 Lower index of toxicity of L-Glu(γ)HXM·VO_3^- relative to that of sodium metavanadate.

Study I: *Daily oral (gavage) administration of $NaVO_3$ and L-Glu(γ)HXM·VO_3^- to healthy CD_1 mice over a period of one week*

Treatment	Dose (mmol/kg/day)	No. of deaths	% of deaths	Notes
$NaVO_3$	0.036	1	12.5	Died day 3
	0.143	2	25	Died day 3
L-Glu(γ)HXM·VO_3^- (2:1)	0.143	0	0	

Study II: *Daily oral administration of L-Glu(γ)HXM·VO_3^- to Wistar male rats over a period of 28 days*

Treatment	Dose	No. of deaths	% of deaths
L-Glu(γ)HXM·VO_3^- (2:1)	Up to 0.3 mmol/kg/d	0	0
	Control untreated, healthy rats	L-Glu(γ)HXM·VO_3^- treated rats (0.3 mmol/kg/d for 28 d)	
Urea (mg/100 ml)	40–50		47 ± 3
Creatinine (mg/100 ml)	0.4–0.7		0.52 ± 0.03
Glutamic-oxaloacetic transaminase (units)	40–80		60 ± 15
Bilirubin (mg/100 ml)	0.23–0.54		0.01 ± 0.002
Alkaline phosphatase (units/liter)	200–600		250 ± 30

Study III: *LD_{50} for intraperitoneal administered sodium metavanadate and for L-Glu(γ)HXM·VO_3^- in male Wistar rats*

Treatment	No.	LD_{50} mmol/kg/d
Sodium metavanadate ($NaVO_3$)	10	0.1 ± 0.02
L-Glu(γ)HXM·VO_3^- (2:1)	10	0.23 ± 0.04

DISCUSSION

We have previously reported that L-Glu(γ)HXM is especially active in synergizing the insulinomimetic efficacy of vanadium. This feature was attributed to the optimal range of the vanadium binding affinity ($K = 1.3$ to $1.9 \times 10^2\ M^{-1}$) and to the efficacy of this particular ligand to associate with the vanadyl(+4) cation and the vanadate(+5) anion, both of which are insulinomimetic [23,24,26,49] with nearly identical affinities. We have therefore suggested that L-Glu(γ)HXM stabilizes the metalooxide in terms of shape, geometry, and coordination modes toward an insulinomimetic active species at the aqueous intracellular milleu of the mammalian cell [31,32].

With the completion of these cell-free and in vitro studies, we investigated here the glucose-lowering potency of L-Glu(γ)HXM·VO_3^- (2:1) in a variety of diabetic rodents, each reflecting a particular aspect of this CHO-intolerance heterogenous disease in humans [43,44]. L-Glu(γ)HXM·VO_3^- was administered exclusively by the oral mode, under nearly identical experimental conditions, and the glu-

cose-lowering pharmacokinetic profiles were studied following either a single administration or daily administrating protocols.

Oral administration of L-Glu(γ)HXM•VO_3^- (2:1) was effective in lowering BGLs in all diabetic rodents studied with no exception. The treatment antagonized hyperglycemia, irrespective of its etiology, i.e., whether it originated from insulin defficiency (e.g., in STZ rats, Figs. 1 and 2), from a decrease in the capability of peripheral tissues to metabolize glucose in response to insulin (e.g., in ZDF male rats, Figs. 4 and 5), or from overproduction of glucose by the liver as in the case of *P. obesus* placed on high-CHO diet (refs. [37,43] and Figs. 6 and 7).

In the single-administrating protocol, doses of 0.14 to 0.28 mmol/kg were found sufficient to obtain a desirable glucose-lowering profile in most diabetic rodents studied. Such profiles are defined by a rapid glucose-lowering pattern ($t_{1/2}$ = 1–3 h in all cases) followed by a prolonged and stable lower or even normal circulating glucose levels over a period of at least 24 h (Figs. 1, 4, and 6). In a daily-administrating protocol, a dose of 0.07 to 0.14 mmol L-Glu(γ)HXM•VO_3^-/kg/d was found appropriate. A characteristic feature of this therapy is the maintenance of lower or normal BGLs for additional 3 to 5 days after cessation of therapy (Figs. 2 and 7).

As shown previously for vanadium therapy in diabetic rodents, the glucose-lowering potency of L-Glu(γ)HXM•VO_3^- therapy is inversely related to the severity of the disease. A profound glucose-lowering effect was obtained in STZ rats 5 days after the induction of the disease (Fig. 1), but only a slight decrease in BGL was obtained 2 weeks after administration of streptozocin (not shown). The same is valid for ZDF male rats and *P. obesus* where therapy-induced normoglycemia at earlier stages of the disease (Figs. 4 and 7) and was less effective in 12-week-old ZDF rats (Fig. 5) or in state-D *P. obesus* (not shown).

The common denominator to 2-week-old STZ rats, 12-week-old ZDF male rats, and state-D *P. obesus* is a drastic depletion in endogeneous insulin reserves [44]. Thus, as suggested for vanadium salts and appears to be valid for L-Glu(γ)HXM•VO_3^- therapy too, vanadium acts in diabetic rodents in vivo as an insulin-sensitizer rather than as an insulin-mimicker [44]. Recently, we have proposed that this sensitizing in vivo effect is in part due to the efficacy of vanadium (but not of insulin) to arrest glucose-6-phosphate (G-6-P) dephosphorylating activity in diabetic peripheral tissues. This in turn normalizes the low levels of G-6-P characterizing diabetic peripheral tissues, thereby enabling further metabolism and/or storage of cell-entered glucose [49].

In several studies performed in human diabetic patients, low vanadium concentrations were used to avoid toxicity (2 ± 1 mg/kg/d, namely, 0.04 mmol/kg body wt/d [33–36]). Although these low doses manifested several antidiabetic effects, extrapolating data from animal studies indicate that higher vanadium concentrations would be significantly more effective in antagonizing the deteriorating effects of diabetes in human patients. L-Glu(γ)HXM•VO_3^- (2:1) is 4–7 times more active than free vanadium in facilitating the mudulating actions of insulin [31,32]. It was therefore of interest to evaluate whether L-Glu(γ)HXM•VO_3^- therapy would be effective within the range of vanadium concentrations currently permitted to be used in humans. As reported here, a daily dose of L-Glu(γ)HXM•VO_3^- at a range of 0.07 to 0.14 mmol/kg/d was effective in lowering BGL in all diabetic rodents studied (Figs. 2, 5, and 7). This value approaches the vanadium dose of 0.04 mmol/kg body wt that is currently allowed for human patients. It is conceivable that even significantly lower doses of L-Glu(γ)HXM•VO_3^- (2:1) are sufficient and appropriate for human diabetic patients, considering the continuous glucose-lowering action obtained after cesation of this therapy (i.e., Figs. 2,7) and the conversion factor needed to account for the higher metabolic rate of rodents vs. that of human. The optimal therapeutic protocol is to be determined experimentally in future studies.

Additional parameters that may encourage future application of L-Glu(γ)HXM•VO_3^- therapy in human diabetic patients include the lower index of toxicity of this combination (Table 1) and the excellent glucose-lowering profile featuring a rapid onset and a prolonged glucose-lowering effect following a single administrating dose (Figs. 1, 3, 4, and 6). Such glucose-lowering pattern differs drastically from that found by various laboratories with regard to free vanadium therapy, where BGL falls at

a slow rate with a $t_{1/2}$ value of 2–3 days, following a daily administration of either free vanadium, or its complexes with maltolate [28] or picolinate [30]. This and several other aspects of L-Glu(γ)HXM·VO_3^- therapy that have been raised in this study are under investigation.

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