The physiological effects of the undercurrent water from Mt. Fuji on type 2 diabetic KK-A^y mice

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Abstract Recently, it has been reported that vanadium exists in a relatively high concentration in the natural water around Mt. Fuji in Japan. Vanadium is known to have a blood glucose lowering effect and improve the diabetic state in human and rodents. Therefore, many researchers have interested in the relationship between diabetes mellitus and vanadium in the undercurrent water from Mt. Fuji. In this study, we examined whether or not 3- or 5-fold concentrated undercurrent water from Mt. Fuji improves hyperglycemia and diabetic state in type 2 diabetic KK-A^y mice with obesity. The concentrated undercurrent water given as drinking water did not reduce high blood glucose level, however, suppressed the progress of obesity, in terms of body weight gain, by the treatment for 12 weeks. Moreover, vanadium was found to accumulate in the tissues such as bone, spleen and liver by the treatment of 5-fold concentrated undercurrent water from Mt. Fuji for 12 weeks.

Keywords: concentrated undercurrent water from Mt. Fuji, type 2 diabetes mellitus, obesity, KK-A^y mice, vanadium.

Introduction

Vanadium, which is an important ultra trace element for mammals, has been known to have some biological and pharmacological properties depending on the oxidation states of the metal [1]. Among them, the insulin-mimetic effect of vanadium compounds has been well documented. In 1899 before the discovery of insulin and its clinical use to treat diabetes mellitus (DM) in 1921, orally administrated sodium vanadate(V) was reported to improve DM in human diabetes [2]. The *in vitro* insulin-mimetic effect of vanadium ions was confirmed later in 1979 [3]. Therefore, the insulin-mimetic effect of vanadium compounds attracted keen interest to the researchers in this field. Following these findings, several research groups attempted to confirm the *in vivo* insulin-mimetic effect of vanadium. The blood glucose level of streptozotocin (STZ)-induced type 1 diabetic rats was found to normalize by addition of sodium vanadate (800 mg/l) in the drinking water in 1985 [4] and 1987 [5]. Moreover, oral sodium vanadate (300 mg/l) in drinking water improved glucose homeostasis in type 2 diabetic *ob/ob* mice [6]. However, most of the glucose lowering effects of vanadium compounds were achieved at high concentrations of vanadium, in which toxic side effects including diarrhea or hepatotoxicity have been noted [5, 7].

Vanadium has been known to widely distribute in natural water such as groundwater, river water, lake water and undercurrent water [8]. In the tap water, undercurrent water and groundwater around Mt. Fuji in Japan contain relatively high concentrations of vanadium [8, 9], the maximum concentration being reported to be approximately 100 μg/l. The long-term administration of vanadium in drinking water at that level of Mt. Fuji groundwater (100 μg/l) has been reported to improve lipid metabolism, but did not lower the high blood glucose levels or improve insulin resistance in type 2 diabetic KK mice [10]. Therefore, we examined whether or not the concentrated undercurrent water from Mt. Fuji improve high blood glucose level, insulin resistance and other diabetic state in type 2 diabetic KK-A^y mice with obesity.

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Experimental

1. Evaluation of antidiabetic effect of the concentrated undercurrent water from Mt. Fuji in KK-A^y mice

Mt. Fuji undercurrent water was collected from the circumference of Fujiyoshida city and concentrated to 3- and 5-fold with respect to the vanadium concentration in the Mt. Fuji Factory of Aoba-Reitoh (Fujiyoshida, Japan). Vanadium concentration in the concentrated undercurrent water was determined by neutron activation analysis (NAA) at the Research Reactor Institute of Kyoto University using the peak area of 1,434.0 keV based on ^{51}V (n, γ) ^{52}V reaction (half-life of ^{52}V: 3.75 min) [11]. KK-A^y mice with type 2 DM were randomly divided into three experimental groups as the control group (Control, n = 5) received tap water, the treated groups received 3- (V: 190 μg/l, n = 5) and 5- (V: 360 μg/l, n = 5) fold concentrated undercurrent water from Mt. Fuji as the drinking water for 12 weeks. Their blood glucose level, body weight, food intake and water consumption were monitored at 2 or 3-day intervals. At the 4 and 12 weeks after

treatment of the concentrated undercurrent water from Mt. Fuji, serum samples for the analyses of urea nitrogen (UN), glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), triglyceride (TG), total cholesterol (TCHO), leptin and insulin levels were obtained from orbital exsanguinations under anesthesia with ether. The serum levels of UN, GPT, GOT, TG and TCHO were measured by a Fuji Dry Chem (Fuji Medical Co., Tokyo, Japan). Serum insulin and leptin levels were determined by Glazyme insulin-EIA test and AN'ALYZA (TECHNE Co., Minneapolis, USA), respectively. Moreover, an oral glucose tolerance test (OGTT) was confirmed. The KK-A^y mice were fasted for 12 hr and glucose at a dose of 1 g/kg body weight was given orally. All blood glucose levels were measured by a glucose oxidase method.

2. Organ distribution of vanadium in KK-A^y mice treated with the concentrated undercurrent water from Mt. Fuji

KK-A^y mice were sacrificed under anesthesia with ether at the 12 weeks after the treatment of the concentrated undercurrent water, and organs such as brain, heart, liver, kidney, spleen, pancreas, adipose, bone and muscle were removed and weighed. The organs were then lyophilized. Vanadium was determined by neutron activation analysis (NAA) [11].

Results and discussion

A previous report demonstrated that vanadium at the concentration of 100 μg/l in drinking water was not able to effect on the glucose metabolism and to improve both glucose tolerance and diabetic status in type 2 diabetic KK mice [10]. Then we examined the effect with the concentrated undercurrent water with high concentrations of vanadium. After the treatment of the mice with the concentrated undercurrent water from Mt. Fuji for 12 weeks, the blood glucose lowering effect and the improvement of insulin resistance in KK-A^y mice were not observed as shown in Fig. 1-A, in which each KK-A^y mouse ingested average vanadium levels of 90 μgV/kg/day (3-fold) or 177 μgV/kg/day (5-fold). In addition, both serum insulin and leptin levels did not significantly change as compared with those of the control KK-A^y mice. However, as shown in Fig. 1-B, the increasing rate of body weight of KK-A^y mice was significantly lowed in KK-A^y mice treated with the 5-fold concentrated undercurrent water. This observation demonstrated that the concentrated undercurrent water suppressed the progression of obesity in type 2 diabetic KK-A^y mice. On the other hand, the food intake and water consumption were not significantly differ between the control and the treated KK-A^y mice during experimental period. Because obesity is deeply associated with the development of insulin resistance and DM in human and rodents [12], the present results suggest that vanadium in the concentrated undercurrent water prevents type 2 DM through reduction of obesity.

In order to evaluate the toxicity by the treatment of the concentrated undercurrent water from Mt. Fuji, we determined the level of UN, GPT and GOT. All serum parameters were not changed from those of the control KK-A^y mice (data not shown). By the treatment with the concentrated undercurrent water neither kidney nor liver function was impaired, as indicated by the parameters of serum UN, GOT and GPT. Moreover, the toxicological symptom such as diarrhea, ataxia, and paralysis of the hind legs were not observed in KK-A^y mice treated with 3- and 5-fold undercurrent water. These results show that the exposure of mice to the low level of vanadium in the drinking water does not impair the hepatic, renal, and other functions. OGTT confirmed that the blood glucose levels in the treated both groups were not significantly different at all time point throughout OGTT as compared with the control KK-A^y mice (data not shown), indicating that the concentrated undercurrent water from Mt. Fuji did not affect to the glucose metabolism by the treatment for 12 weeks.

Vanadium concentrations in the tissues after the treatment of 5-fold undercurrent water for 12 weeks were determined by NAA. Interestingly, vanadium accumulation in the tissues such as heart, liver, kidney, spleen, pancreas, adipose, bone and muscle was observed (Table 1). In particular, vanadium concentrations in the

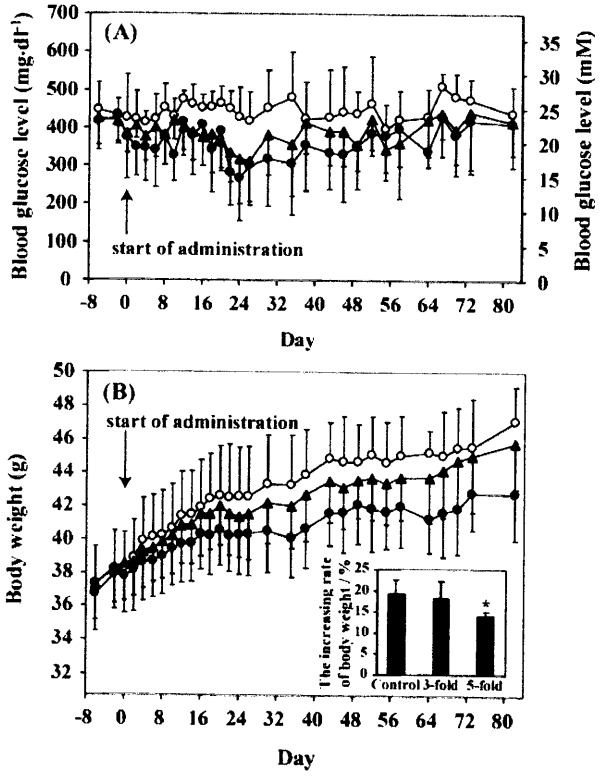

Figure 1. Changes of blood glucose level (A) and body weight (B) in the control KK-A^y mice and KK-A^y mice treated with the concentrated undercurrent water from Mt. Fuji as a drinking water. ○, control KK-A^y mice; ▲, KK-A^y mice treated with 3-fold concentrated Mt. Fuji undercurrent water; ●, KK-A^y mice treated with 5-fold concentrated Mt. Fuji undercurrent water.
Each symbol is expressed as the mean value ± SD (n = 5).
Inset in (B): the increasing rate of body weight in KK-A^y mice.
*Significance at $P < 0.05$ *vs.* the control KK-A^y mice.

Table 1
Organ distribution of vanadium in KK-A^y mice treated with 5-fold concentrated undercurrent water from Mt. Fuji for 12 weeks

Organ	Control	5-fold concentrated water
	ng/g of wet weight	ng/g of wet weight
Bone	n.d.	442 ± 150
Spleen	n.d.	254 ± 73
Liver	n.d.	142 ± 17
Kidney	n.d.	87 ± 26
Heart	n.d.	56 ± 18
Adipose	n.d.	50 ± 9
Muscle	n.d.	32 ± 5
Pancreas	n.d.	29 ± 8
Brain	n.d.	n.d.

Data are expressed as the mean values ± SD (n = 5)
n.d.= not detected

bone, spleen and liver were significantly increased. When the blood glucose level was lowered with vanadium ions, the dose of vanadium at least 10 mgV/kg body weight of animal/day by oral administration is need [11, 13-15]. Therefore, the intake of vanadium at the dose of 177 μgV/kg/day in the present examination corresponds to approximately 1/60 of $VOSO_4$ administration. On the other hand, vanadium clearance from the blood of rats given $VOSO_4$ by *i.p.* injection has been reported to be very quick (CL_{tot} = 38.9 ± 5.8 $ml \cdot min^{-1} \cdot kg^{-1}$) [16]. Nevertheless, vanadium accumulation in several organs of the mice was found after 12 weeks. When the blood glucose level was lowered with vanadium compounds without toxicity, the vanadium concentrations in the organs increased in 10-30 times higher than those of KK-A^y mice treated with 5-fold undercurrent water [17]. These facts suggest that prolongation of the water supply brings about the blood glucose lowering effect in the mice.

In conclusion, 5-fold concentrated undercurrent water from Mt. Fuji suppressed the progression of obesity without remarkable toxicity in type 2 diabetic mice. The administration of the 5-fold concentrated undercurrent water caused accumulation of vanadium in several organs of the mice. From these results, it is suggested that a longer-term intake of the concentrated undercurrent water from Mt. Fuji improves the diabetic state in KK-A^y mice.

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