EFFECTS OF GINSENG COMPONENTS ON RODENTICIDE VACOR-INDUCED DIABETES MELLITUS IN RATS

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ABSTRACT

It is now well established that the rodenticide Vacor (N-3-pyridyl-mehtyl-N'-p-nitrophenylurea) causes a hyperglycemia in human and rats. It is also reported that there are some components (DPG-3) in ginseng radix which cause hypoglycemic effect on alloxan diabetic mice. In the present study, attempts were made to demonstrate in Vacor-poisoned rats the hypoglycemic activity of red ginseng component (RGC), which was extracted by Kimura's DPG-3 extraction procedure and found to be effective for lowering a hyperglycemia in alloxan-diabetic rats.

Vacor in a dose of LD_{50} (10mg/kg) produced a glucose intolerance with a paradoxical moderate increase in blood immunoreactive insulin and derangement in glucose metabolism of epididymal adipocytes in rats. Although RGC (20mg/kg, i.p.) did not exert any significant influence on a hyperglycemia induced by large lethal doses (25mg/kg) of Vacor ingestion, it improved the LD_{50} Vacor-induced glucose intolerance and caused a further increase in blood insulin levels in Vacor-poisoned rats. The administration of RGC (20mg/kg, i.p.) normalized Vacor-induced depression of glucose metabolism and lipogenesis in the epididymal adipocytes with an improvement of reduced responses to insulin of adipocytes from Vacor-poisoned rats. These results suggest that some red ginsneng components contained in RGC fraction normalize the depressed peripheral glucose unitlization and insulin response and eventually lead to an improvement of abnormal glucose tolerance developed in rats poisoned with small doses of Vacor.

INTRODUCTION

N-3-Pyridylmethyl-N'-p-nitrophenylurea, or Vacor (RH-787) was introduced in 1974 as a rat poison, which would kill even Wafarin-resistant rats but not harm other animal life (1). The LD_{50} of Vacor is very low for rats (6.7mg/kg) (2) but quite high for nontarget species, including dogs, cats, and chickens. Unfortunately, Vacor poisoning in man occurred after accidental and suicidal ingestion of the compound (3,4). The survivors of Vacor ingestion develop ketoacidosis-prone diabetes mellitus and severe toxic neuropathies (5).

The Vacor-induced diabetes is now generally believed to be caused by the destruction of B cells in the pancreatic islets of Langerhans, resulting in the development of insulin-dependent diabetes (6). However, there are a number of evidence indicating that it is not insulin-dependent and some other extra-pancreatic factors are involved in its pathogenesis. For example, Lee et al (7, 8) demonstrated that Vacor-poisoned patients and rats showed a normal insulin and C-peptide secretory responses to oral glucose loading despite the development of hyperglyce-

mia.

The beneficial effects of *Panax ginseng* on diabetes mellitus are now well documented (9), and it has recently been described that a non-saponin component of ginseng radix is effective for lowering hyperglycemia in alloxan diabetic mcie by stimulating insulin release from pancreatic islets (10, 11). In the present study, we have tried to demonstrate a hypoglycemic activity of red ginseng component(s) in Vacor-poisoned rats.

MATERIALS AND METHODS

Animals. Wistar strain rats of both sexes weighing 200-250g were used in observing the changes in blood glucose level, and young male rats weighing 100-150g were used for the experiments with adipocytes. The animals were faster for about 15 hr before each experiment.

Ginseng Extracts. Red ginseng radix (*Panax ginseng* C.A. Meyer) supplied by Korea Ginseng & Tobacco Research Institute was finely powdered and suspended in 9 weight-volumes of water. The suspension was shaken for 36 hr at 4°C and centrifuged at 1,000 x g for 10 min. The resulting supernatant solution was used as the water extract of red ginseng. The water extract was further fractionated as described by Kimura et al (10) to obtain RGC fraction, which corresponds to the DPG-3 fraction of Kimura et al (10). The water extract was given to animals by gastric intubation, and the RGC fraction in isotonic saline solution by intraperitoneal injection (i.p.).

Vacor. Vacor (Rohm & Haas Co., Pa) was suspended in 0.05% Tween 80 solution and given to animals by gastric incubation at a dose of 5-25mg/kg body weight.

Alloxan Diabetes. Alloxan monohydrate (40mg/kg) dissolved in 0.9% saline solution was injected into a tail vein of rats weighing 200-250g. Two days after the injection, blood glucose levels were determined at fasting state, and the rats with a glucose level of 150mg% or more were used as alloxan diabetics.

Assay for Blood Glucose and Insulin Levels. Blood samples were obrained from tail veins. The glucose level was determined by glucose oxidase-peroxidase method (12), and the insulin level as immuno-reactive insulin (IRI) by the radioimmunoassay using double antibody (13).

Assay for (1-^{14}C) Glucose Conversion to $^{14}CO_2$ and ^{14}C-Lipids. Rats were killed by a blow on the head, and the epididymal fat pads were quickly removed. The adipocytes were prepared from the these fat pads as described by Rodbell 14). The adipocytes (8 x 10^4 cells) were incubated at 37°C for 2 hr in 2 ml of bicarbonate-buffered medium containing albumin (36mg/ml), 0.5mM glucose, 0.1μCi of (1-^{14}C) glucose, and insulin (pork monocomponent insulin, Novo Research Institute, Copenhagen) as described by Weiss and Loeffler (15). After incubation, 1ml of 8N H_2SO_4 was added to the incubation medium after which the $^{14}CO_2$ was trapped on filter paper impregnated with ethanolamine: methylcellosolve (1:2 v/v). For the determination of ^{14}C-lipid, the cell suspensions were extracted with 5ml of Dole's extraction mixture as described by Rodbell (14), and the lipid fraction was assayed for ^{14}C radioactivity.

Radioactivity Assay. ^{125}I radioactivity was counted in a Beckman gamma counter. ^{14}C radioactivity was counted in a Packard Tri-Carb 300C liquid-scintillation system, using Jeffay & Alvarez scintillator solution containing PPO and dimethyl POPOP (16).

RESULTS AND DISCUSSION

Effect on Vacor-Induced Hyperglycemia. Single large dose (25mg/kg) of Vacor, which had previously been employed in experimental Vacor poisoning,[7,8] caused half of the animals to die around 6 hr after Vacor administration, and hyperglycemia occurred only with imminent death of the animals. As shown in Table I, the red ginseng water extract (30mg/kg per os) and RGC fraction (20mg/kg i.p.) given every other day for 10 to 20 days prior to Vacor administration did not exert any influence on Vacor-induced hyperglycemia, nor did they prolong the life of Vacor-poisoned rats. On the other hand, the RGC fraction given twice in a dose of 20mg/kg to

Table 1. Effect of red ginseng pre-treatment of rats on changes in blood glucose level induced by Vacor poisoning

Red ginseng extracts were given every other day for 10 and 20 days before Vacor (25mg/kg) poisoning. Fasting blood levels were determined at a given time after Vacor administration.

Pre-treatment with	Blood glucose level after Vacor at			
	0	0	4	6 hrs
	mg/100ml			
1. None (control)	68 ± 3 (10)*	76 ± 6 (10)	98 ± 10 (8)	180 ± 17 (4)
2. Water extract (30mg/kg, orally)				
For 10 days	75 ± 5 (10)	80 ± 6 (10)	102 ± 10 (8)	188 ± 16 (5)
For 20 days	75 ± 6 (10)	82 ± 7 (9)	100 ± 12 (7)	184 ± 12 (4)
3. RGC (20mg/kg, i.p.)				
For 10 days	65 ± 7 (10)	75 ± 7 (10)	110 ± 9 (8)	180 ± 15 (5)
For 20 days	71 ± 7 (10)	75 ± 5 (10)	105 ± 8 (9)	168 ± 18 (6)
With Vacor simultaneously	79 ± 6 (5)	73 ± 8 (5)	98 ± 9 (4)	174 ± 15 (3)

Values are mean ± S.E. Figures in parentheses are the number of animals.

alloxan diabetic rats were proved to be effective for alloxan-induced hyperglycemia (Table II). Thus, the ineffectiveness of red ginseng extracts for Vacor-induced hyperglycemia might be primarily due to the early death of the animals poisoned with large dose of Vacor. For this reason, in the following experiments the doses of Vacor were reduced to 5 to 10mg/kg, which are close to LD_{50} (2), to prolong the animal life after Vacor poisoning.

Effect on Glucose Intolerance Induced by Small Dosea of Vacor. Small doses (5 to 10mg/kg) of Vacor did not kill rats nor caused hyperglycemia to occur in earlier times after Vacor administration as in the case of large doses of Vacor poisoning. Oral glucose tolerance test, however, revealed that Vacor-poisoned rats developed a glucose intolerance. As shown in Fig. 1, the repeated administration of red ginseng RGC fraction for prolonged time improved the glucose intolerance in Vacor-poisoned rats. Futhermore, as indicated by the number of rats survived, the RGC fraction reduced the death rate of Vacor-poisoned rats.

Effect on Blood Insulin Levles. As shown in Table III, both Vacor and RGC fraction produced a moderate but insignificant increase in blood IRI levels. When rats were pre-treated with RGC fraction (10 and 20mg/kg) and then poisoned with Vacor (10mg/kg) 3 hr later, a further in-

Table 2. Effects of ginseng extracts on alloxan-induced hyperglycemia in rats

The ginseng extracts were given twice 49 and 99 hrs after alloxan (40mg/kg, i.v.) injection.

Ginseng extract	Time after 1st ginseng administration		
	0	40	72 hr*
	Blood glucose (mg%)		
None (control)	168.4 ± 34.9**	191.2 ± 15.4	258.2 ± 63.1
Water extract (30mg/kg, p.o.)	181.6 ± 26.4	218.4 ± 34.0[a]	283.6 ± 48.0[b]
RGC fraction (02mg/kg, i.p.)	176.0 ± 32.1	150.0 ± 44.5[c]	151.4 ± 36.1[d]

* Corresponds to 22 hrs after the second ginseng administration.

** Figures are mean ± SD. n=14 for control and RGC groups, and n=15 for water extract gorup.

a and c, $p<0.05$ compared to control; b, $p<0.1$ compared to control; d, $p<0.01$ compared to control.

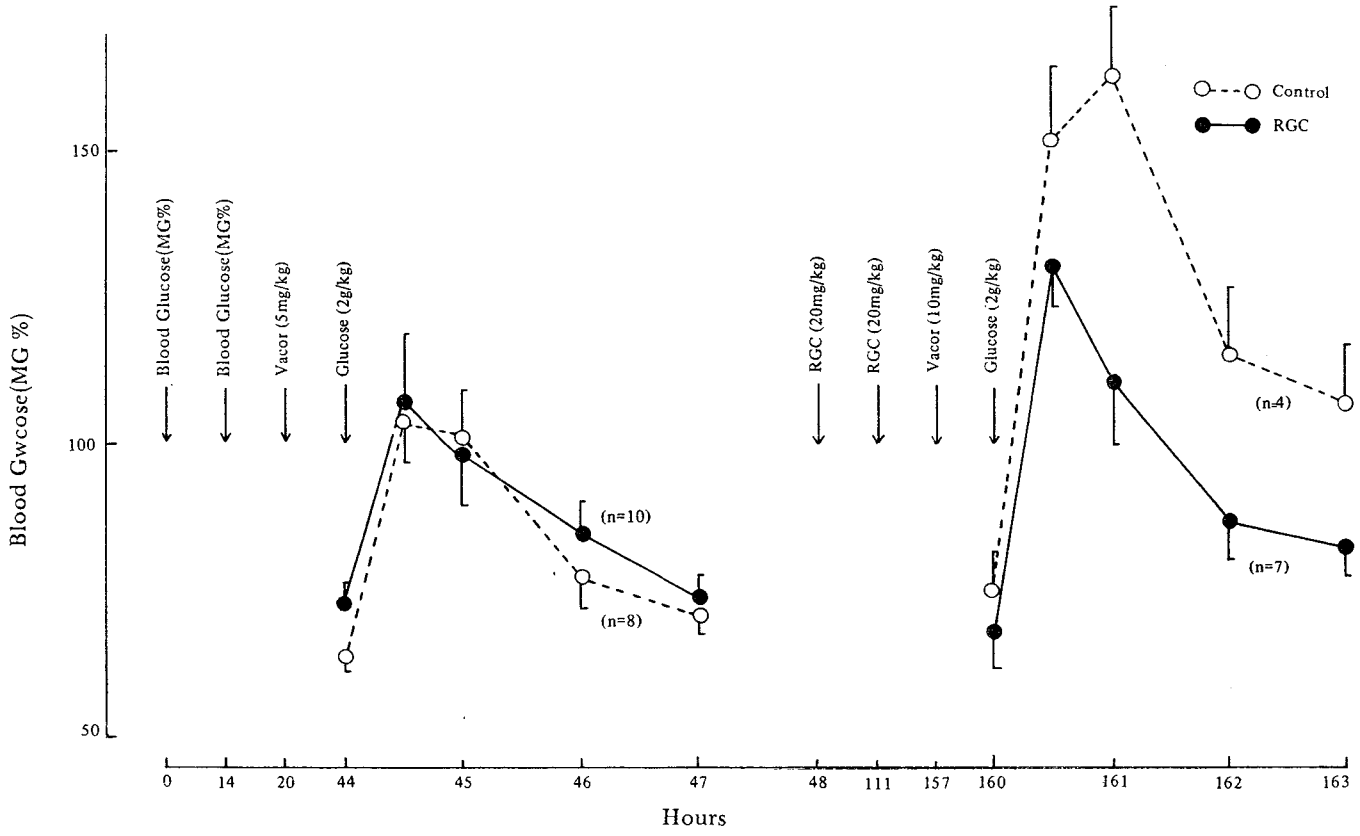

Fig. 1. Effect of ginseng RGC fraction on oral glucose tolerance test in Vacor-poisoned rats. Control rats received only Vacor at the time indicated by arrow sign for Vacor. RGC-treated rats received both Vacor and RGC at the time indicated by arrow signs for each agent. Values are mean ± SD.

Table 3. Effect of RGC fraction on blood insulin levles in normal and Vacor-poisoned rats

The RGC fraction (10 and 20mg/kg) was given intraperitoneally 3 hrs before Vacor (10mg/kg) poisoning. Blood immunoreactive insulin (IRI) was assayed 2 hrs after Vacor administration.

Dose of RGC	Blood IRI level	
	Normal	Vacor poisoned
mg/kg	uU/ml	
0	23.2 ± 3.8	35.0 ± 8.3[a]
10	35.6 ± 7.6[b]	56.2 ± 10.7[c]
20	34.1 ± 8.2	68.5 ± 13.4[d]

Each value represents mean ± S.E. from 5 rats.

[a] $p < 0.3$ compared to normal controls.

[b] $p < 0.2$ compared to normal control without RGC treatment.

[c] $p < 0.2$ compared to Vacor-poisoned control without RGC.

[d] $p < 0.1$ compared to normal with 20mg/kg RGC, and to Vacor control without RGC.

crease in blood IRI levels was noted 2 hr after Vacor administration. These results indicate that the insulin release from the pancreas in stimulated rather than depressed by Vacor and that both Vacor and RGC fraction act synergistically in stimulating the pancreatic insulin secretion.

Effects on Glucose Metabolism in Adipocytes. Vacor given in a dose of 10mg/kg 3 hr before produced a small but significant decrease in the metabolic conversion of (1-^{14}C) glucose to $^{14}CO_2$ and ^{14}C-lipid by rat epididymal adipocytes, whereas the RGC fraction per se had no effect in normal adipocytes. However, the RGC fraction, when given to Vacor-poisoned rats in a dose of 20mg/kg 2 hr after Vacor posisoning, normalized the decreased rate of glucose metabolism in Vocorpoisoned adipocytes (Table IV).

Insulin (0.1mU/ml) added *in vitro* to normal adipocyte suspension produced a significant increase in the conversion of glucose carbon to CO_2 and lipid, and these metabolic response to insulin of adipocytes were also depressed by Vacor poisoning. The RGC fraction which had no effect on these normal responses to insulin increased to some extent the reduced responses to insulin of adipocytes from Vacor-poisoned rats (Table IV).

Taken altogether, these results suggest that the glucose intolerance developed in rats poisoned with small doses of Vacor is not primarily due to an insulin deficiency and that the improvement of glucose intolerance by the RGC fraction is ascribable to the normalization of the depressed peripheral glucose utilization in Vacor-poisoned rats.

Table 4. Effect of RGC treatment on insulin added in vitro on rates of conversion of (1-^{14}C) glucose to $^{14}CO_2$ and ^{14}C-lipid by adipocytes of Vacor-poisoned rats

Rats were poisoned with Vacor (10mg/kg) 2 hr prior to RGC (20mg/kg) treatment. One hr after RGC treatment, rats were sacrificed. Isulin, where present, was at a concentration of 0.1mU/ml.

Animals	Relative rate of (1-^{14}C) glucose → $^{14}CO_2$		Relative rate of (1-^{14}C) glucose → ^{14}C-lipid	
	− Insulin	+ Insulin	− Insulin	+ Insulin
Control	100	158 ± 7.4[a]	100	136 ± 6.4[a]
Vacor-poisoned	88 ± 3.6[b]	119 ± 4.4	91 ± 3.5[b]	118 ∓ 5.5
Control + RGC	103 ± 3.7	145 ± 5.2	99 ± 4.2	130 ± 4.8
Vacor + RGC	98 ± 3.8[c]	128 ± 4.6[d]	105 ± 3.8[e]	125 ± 6.2[f]

In control without insulin addition, the rate of (1-^{14}C) glucose conversion to $^{14}CO_2$ was 3.1 ± 1.1 μmoles/10^7 cells/2 hrs, and that to ^{14}C-lipid was 3.3 ± 0.15 μmoles/10^7 cells/2 hrs. These control values were normalized to 100; all other values were expressed relative to the control. Results are reported as mean ± S.E. from 5 rats.
[a]$p < 0.001$ compared to no addition of insulin; [b]$p < 0.02$ compared to control without insulin; [c]$p < 0.1$ against Vacor-rats; $d_p < 0.001$ against no insulin group; [e]$p < 0.15$ against Vacor-control. $f_p < 0.02$ against no insulin group of (Vacor + RGC).

Chong: Could you explain me why you used that rodenticide? Because it sounds rather unphysiological for me to induce hyperglycemia. Rodenticide could be hepatotoxic agent causing epinephric effects.

Lee: Some other hormonal unbalance maybe caused by the rodenticide, vacor. That effect can't be neglected. We had examined some glucagon secreatory response after vacor administration, but the glucagon secreation was normal, not impaired.

Chong: Sure. But, did you look at the liver on histology after the rats were sacrificed? What did they show? Dit it show hepatic necrosis which could explain for the glucose intolerance you observed?

Lee: We examined the autopsy sample. There was some degenerational change in the liver of the patients, but not in vacor-poisoned animals.

Chong: Thank you.

인삼성분이 살서제(Vacor)로 유발시킨 쥐의 당뇨에 미치는 영향

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고려홍삼에서 추출한 성분(RGF-3)이 살서제 Vacor로 유발된 당뇨병에 어떤 영향을 미치는가를 흰쥐에서 관찰하였다.

홍삼 RGF-3 분획의 복강내투여(20mg/kg)는 alloxan-유발고혈당을 저하시키고 소량(10mg/kg)의 Vacor로 유발된 당불내성을 개선시켰다.

소량의 Vacor로 중독된 흰쥐는 정상대조군에 비하여 높은 혈중 insulin치를 나타내는 경향이었으며, Vacor투여 3시간 전에 복강내 투여한 RGF-3은 정상대조군에 있어서보다 더 높은 혈중 insulin치의 증가를 가져왔다.

흰쥐의 부고환지방세포의 glucose대사〔(1－^{14}C)glucose의 $^{14}CO_2$에로의 전환〕와 glucose로부터의 지방합성〔(1－^{14}C) glucose로부터의 ^{14}C-lipid에로의 전환〕은 Vacor중독에 의하여 저하되었으나, RGF-3 투여에 의하여 정상수준까지 회복되었으나, 그러나 정상지방세포에 대해서는 RGF-3은 별다른 영향을 주지 아니하였다.

한편 이상과 같은 말초지방세포의 glucose 대사는 insulin의 in vitro 첨가에 의하여 촉진되었으나,Vacor 중독에 의하여 감약되었고, RGF-3은 Vacor 중독에 의한 감약된 반응을 어느정도 회복시켰다.

홍삼의 수침추출물(30mg/kg)과 RGF-3분획(20mg/kg)의 10～20일간 격일 투여는 그 후 대량(25mg/kg)의 Vacor 중독으로 유발되는 고혈당 발생에는 별 영향을 주지 아니하였다.

이와 같은 실험결과에 비추어 홍삼의 RGF-3 분획은 Vacor 중독으로 저하된 말초조직의 당대사를 개선시켜 소량의 Vacor 중독시의 당불내성을 정상으로 회복시키는 것이라 사료되었다.

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