Studies on the Hypoglycemic Effect of Ginseng Polypeptide

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Abstract□ The ginseng polypeptide (GPP) isolated from the root of *Panax ginseng* C.A. Meyer was demonstrated to decrease the levels of blood sugar and hepatic glycogen when injected intravenously to rats at a doses of 50-200 mg/kg without affecting blood total lipid. When mice were injected subcutaneously daily at a dose of 50 and 100 mg/kg for 7 successive days, GPP was also found to decrease blood sugar and hepatic glycogen. In addition, GPP was found to decrease various experimental hyperglycemias induced by injection of adrenaline, glucose and alloxan. GPP exhibited inhibiting effect on the glycogen enhancement induced by glucose, but strenthening effect on the glycogen decrease induced by adrenaline. When the levels of blood total lipid and liver glycogen were increased by alloxan, GPP was shown to inhibit these changes except its lowering blood sugar. The toxicity of GPP is very low, its LD_{50} was found to be 1.62 ± 0.130 g/kg for iv.

Keywords□ Ginseng polypeptide (GPP), blood sugar, hepatic glycogen.

Ando *et al.*[1] had reported that a polypeptide isolated from ginseng roots, which consists of 14 amine acids, showed inhibiting effect on lipolysis induced by adrenaline. Recently, Changjing *et al.*[2] reported that a new polypeptide was isolated from ginseng roots. The chemical structure of the peptide is slighly different from that reported by Ando *et al.* and the sequence of amine acids in the ginseng polypeptide is as follows:

Glu-Thr-Val-Glu-Ile-Ile-Asp-Ser-Glu-Gly-Gly -Gly-Asp-Ala
1 5 10 14

In the present study, we investigated the effect of the polypeptide on the carbohydrate metabolism.

Materials and Methods

Animals: Male Wistar rats weighing 150-160 g and male Ku-Ming strain mice weighing 20-22 g were used in a series of experiments. They were maintained at 23 ± 1 °C under an alternating 12 h light/dark cycle, and given standard laboratory chow and fresh water ad libitum.

Ginseng Polypeptide(GPP)

GPP was isolated from Jilin white ginseng by Prof. Changjing, laboratory of Enzyme Engineering, Jilin university. GPP is white powder and easy to dissolve in water. GPP was made up into the required concentration with physiological saline just before doing experiment.

Determination of blood sugar, hepatic glycogen and blood total lipid contents

o-Toluidine, iodine and vanilin reagents were employed for the determination of blood sugar[3], hepatic glycogen[4] and blood total lipid contents[5], respectively, in the samples.

Data analysis

The values are the means ± S.D. The significance of differences vs. the control group was determined by applying student's t test, and is indicated as follows: $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p$ 0.001.

Results

Effect of once intravenous injection of GPP on

blood sugar, total lipid and hepatic glycogen contents in normal rat

Thirty-two rats, which were fasted for 14 h, were equally divided into 4 groups and injected intravenously once GPP according to the doses in Table 1. The control group rats were given saline 10 ml/kg. At 2 h after injection, they were sacrificed by decapitation, and blood as well liver were rapidly obtained to measure blood sugar, total lipid and glycogen contents.

The data in Table 1 show that the blood sugar and total lipid contents were dose-dependently decreased by the injection with GPP, but blood total lipid content showed no significant change.

Effect of repeated injection of GPP on the blood sugar, total lipid and hepatic glycogen contents in mice

Thirty-two mice were divided into 4 groups and one group was subjected to a subcutaneous injection with saline (10 ml/kg/d) for 7 successive days as the control. The other 3 groups of mice were treated with GPP at a dose of 50, 100 and 200 mg/kg/d for the same period respectively. At 1 h after fourth injection, blood sample was taken from orbital vein to measure blood sugar content. At 1 h after last injection, all mice were killed by decapitation and portion of liver and blood were quickly sampled to measure liver glycogen, blood sugar and total lipid contents.

As shown in Table 2, the repeated subcutaneous injection of GPP produced likewise the decrease of blood sugar and liver glycogen contents in dose-dependent manner, but the level of blood lipid was not markedly affected.

Table 1. Effect of intravenous injection of GPP on blood sugar, total lipid and hepatic glycogen contents in rats

Group mg/kg	Blood sugar mmol/l	B.T.L. mg/dl	Hepatic glycogen mg/100 mg WLT
Control	5.91±0.727	4.01±0.637	1.36±0.844
GPP 50	4.85±0.606**	3.72±0.891	0.86±0.598
100	4.60±0.929**	3.64±0.960	0.79±0.647
200	4.44±0.970**	3.91±0.993	0.50±0.469*

*and **: see data analysis. WLT = Wet liver tissue. B.T.L = Blood total lipid.

Effect of GPP on the hyperglycemia induced by adrenaline in mice

Forty-eight mice were equally divided into 3 groups. Two out of the 3 groups were injected subcutaneously saline 10 ml/kg, and the residual group was injected GPP at a dose of 100 mg/kg for 7 successive days. At 20 min after the final injection, one saline- and GPP-treated group were injected intraperitoneally adrenaline 50 μg/kg, and the residual saline-treated group was injected saline 2 ml/kg. Half the number of mice in each group were decapitated at 2 and 30 min after injection of adrenaline, and liver and blood were obtained to measure blood sugar, total lipid and glycogen contents.

The data in Table 3 show that GPP was able to decrease the hyperglycemia and strengthen glycogen decrease induced by adrenaline respectively. However, they all showed no significant affect on blood lipid content.

Table 2. Effect of repeated subcutaneous of GPP on blood sugar, total lipid and hepatic glycogen contents in mice

Group mg/kg	Blood sugar (mmol/l) 4d[a]	Blood sugar (mmol/l) 7d	B.T.L mg%	Hepatic glycogen mg/100 mg WLT
Control	5.46±0.677	5.46±1.116	464±98.2	5.72±1.427
GPP 50	4.70±0.818*	5.25±0.729	462±64.9	3.62±1.584*
100	4.25±0.556*	4.36±0.719*	449±72.9	2.25±0.911***
200	3.85±0.350**	3.76±0.510**	451±63.5	2.01±0.515***

*, ** and ***: See data analysis; a) = Days after administration; B.T.L = Blood total lipid; WLT = Wet liver tissue.

Table 3. Effect of GPP on adrenaline-induced hyperglycemia in mice

Group	Blood sugar (mmol/*l*)		BTL (mg%)		Liver glycogen (mg%)	
	2[a]	30	2	30	2	30
Control	5.91±0.851	5.91±1.212	698±89	619±126	3.58±0.575	1.93±0.860
Adr. 50 μg/kg	5.86±1.566	6.77±1.364	592±96	621±129	2.41±0.508***	1.07±0.579*
GPP 100 mg/kg+ Adr. 50 μg/kg	5.15±1.465	4.65±0.808##	527±47	649±219	1.49±0.812***#	0.58±0.264***#

*p<0.05, ***p<0.001 as compared with control group; #p<0.05, ##p<0.01 as compared with Adr. group; a=time (minute) after injection of adrenaline; Adr.=Adrenaline; BTL=Blood total lipid

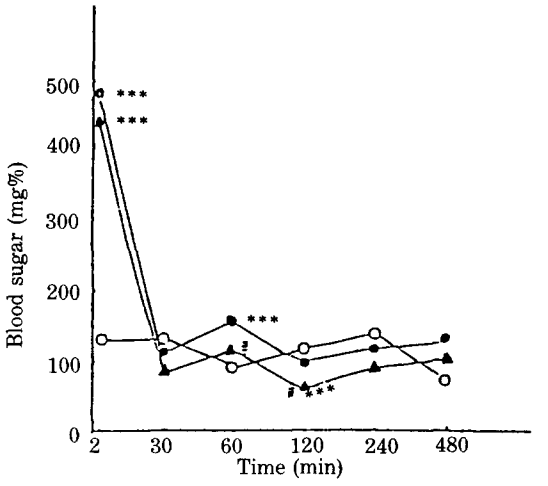

Fig. 1. Effect of ginseng polypeptide on the hyperglycemia induced by injection of glucose in mice. ○–○ Control; ●–● Glucose 2g/kg; ▲–▲ Ginseng polypeptide 100 mg/kg+glucose 2g/kg.
**p<0.01, ***p<0.001 vs control; #p<0.05 vs glucose.

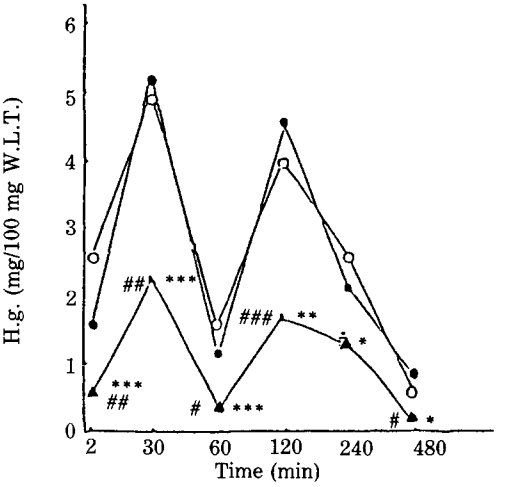

Fig. 2. Effect of ginseng polypeptide on the content of hepatic glycogen after injection of glucose in mice. H.G.=Hepatic glycogen; W.L.T=Wet liver tissue. ○—○ Control; ●—● Glucose 2 g/kg; ▲—▲ Ginseng polypeptide 100 mg/kg+ glucose 2 g/kg.
*p<0.05, **p<0.01, ***p<0.001 vs control; #p<0.05, ##p<0.01, ###p<0.001 vs glucose.

Effect of GPP on hyperglycemia induced by glucose in mice

One hundred and forty-four mice were equally divided into 3 groups. Two out of 3 groups were injected subcutaneously saline 10 ml/kg and the residual group was injected GPP at a dose of 100 mg/kg for 6 successive days. Mice of one saline- and GPP-treated groups were immediately injected intravenously glucose 2 g/kg, and the residual group was injected saline 5 ml/kg after the last administration of GPP. At 2-480 min after administration of glucose, 8 mice from each group were decapitated to measure blood sugar and liver glycogen contents.

From Fig. 1 and 2, it was found that the repeatedly subcutaneous injection of GPP exerted inhibiting effect on hyperglycemia and glycogen increase induced by administration of glucose. The hypoglycemic action of GPP was most significant at 2 h after injection of glucose. However, its glycogen-lowering action was obvious in the whole observation course.

Effect of GPP on hyperglycemia induced by alloxan in rats

Hyperglycemic rats were prepared by intrave-

nous administration of alloxan at a dose of 70 mg/kg after overnight starvation. After 5 days of the alloxan treatment, hyperglycemic rats were selected (plasma glucose level 250-500 mg/dl) and used. Forty-eight hyperglycemic rats were equally divided into 4 groups: alloxan-intoxicated control group, insulin-treated group and two GPP-treated groups, and another 12 non-intoxicated rats were used as normal control group. Rats of normal group and intoxicated control group were injected subcutaneously saline (5 ml/kg/d) and 3 treated groups were injected respectively insulin (1 u/kg/d) or GPP(100 and 200 mg/kg/d) since sixth day of intoxication. At 1 h after administration of GPP on eighth and eleventh days of intoxication, blood sample was taken from tail vein to measure blood sugar content. Thereafter administration of GPP was stopped. At fourteenth and seventeenth days after intoxication, blood sugar was measured once, respectively. Administration carried out again since eighteenth day of intoxication and at 1 h after injection of GPP on twenty-one day of intoxication, all rats were decapitated and blood sugar and liver glycogen contents were measured.

From Table 4, it was found that repeatedly subcutaneous injectio of GPP produced significant and cutaneous injection to GPP produced significant and by alloxan. When administration was stopped, blood sugar level in treated groups gradually enhanced, once more administration of GPP and insulin produced decreases of blood sugar and glycogen contents again. Though both GPP and insulin all were able to decrease blood sugar, their affect on liver glycogen was different from each other. The former caused liver glycogen to increase, but the latter did it to decrease. It was obvious that effects of GPP and insulin on carbohydrate metabolism are not completely identical.

Acute toxicity

When administered intravenously and observed for 72 hours, LD_{50} of GPP for mice was found to be 1.62 ± 0.130 g/kg.

Discussion

Ginseng polypeptide is isolated from the roots of *Panax ginseng* of Jiling origin of China, its chemical structure was established and shown to possess 14 residues[2]. The present experiment demonstrated that GPP exhibits hypoglycemic action against normal blood sugar and various experimental hyperglycemia whether subcutaneous or intravenous injection. When GPP caused hypoglycemia, liver glycogen content was decreased, but blood total lipid was not significant change. Because GPP produced simultaneously hypoglycemia and decrease of glycogen, Obviously, its affect on carbohydrate metabolism is different from that of insulin. As to the mechanism of hypoglycemia of GPP, we have

Table 4. Effect of GPP on blood sugar and liver glycogen in rat intoxicated with alloxan

Group mg/kg	Blood sugar (mmol/l)							Liver glycogen (mg/100 mg WLT)
	4[a]	5	8	11	14	17	21	
Control	5.7±0.38	5.6±0.52	5.3±1.16	5.5±0.37	5.6±0.72	5.2±0.53	5.6±1.11	4.95±1.39
Alloxan 70	*** 20±5.6	*** 23±10.3	*** 19±4.7	*** 23±4.9	*** 19±6.3	*** 20±7.4	*** 19±8.5	5.95±1.88
Ins.1 u/kg+ Alloxan 70	*** 20±7.8	*** 22±11.5	### 6.5±1.43	**### 8.9±3.09	*** 16±9.1	***## 13±5.4	*### 10±5.85	* 6.81±2.23
GPP 200+ Alloxan 70	*** 18±9.2	*** 19±6.8	### 7.3±3.01	***### 11±3.9	*** 21±7.7	*** 19±12.3	**### 12±6.05	***## 2.27±1.84
GPP 100+ Alloxan 70	*** 19±7.1	*** 20±5.9	*### 8.5±3.32	***### 13±5.5	*** 18±6.4	*** 22±9.0	***# 17±2.65	**## 2.57±1.88

*p<0.05, **p<0.01, ***p<0.001 as compared with control group; #p<0.05, ##p<0.01, ###p<0.001 as compared with alloxan group; a=Times (days) after injection of alloxan. Ins.=Insuline; WLT=Wet liver tissue

made further research and will reported in next paper.

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