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Effect of Ginseng Saponin on Alcohol Metabolism in the Animal Body

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Abstract□Unlike carbohydrate and fats, alcohol is essentially foreign to the body and it is known that the body get rid of it by oxidizing alcohol maily in the liver. Acetaldehyde is produced during ethanol metabolism and is known to be oxidized mainly by aldehyde dehydrogenase (ALDH). ALDH activity was found mainly in the mitochondrial fraction but a significant ALDH activity was also present in microsomal and cytosol fraction. Wistar rats (150~200 g, male) were given freely with 12% ethanol (Control) and/or 12% ethanol containing 0.1% ginseng saponins (Test) instead of water for 6 days and the liver was analyzed. ALDH activities of both control and test group were lower than that of normal group but test ALDH was less inhibited than control. ADH activies of both control and test were slightly higher than that of normal group but our previous data showed that it became gradually steady after prolonged ethanol feeding. MEOS activities of both control and test group were much higher than that of normal group. MEOS enzymes are inducible but the activity of test group was greatly higher than that of control. Ethanol containing [1-^{14}C] ethanol (5 μCi) was injected to the above three groups and 30 min later, the distribution of radioactivity of hepatic lipids was investigated. Radioactivities of hepatic lipids of both control and test group were higher than that of normal group, however, that of test group was much lower than that of control. Analysis of individual lipids showed that phospholipid biosynthesis was significantly impaired and fatty acid and triglycerides biosynthesis were greatly stimulated. However, it was realized that the saponin prevented phospholipid biosynthesis depression and the increase of triglyceride biosynthesis considerably. It seemed that the saponin might stimulate ADH, ALDH and MEOS and the acetaldehyde formed would be removed faster. The excess hydrogen can be shunt more quickly into lipid biosynthesis. Electron microscopic observation showed that the hepatic cell of control group was significantly damaged. Mitochondria were swollen and rough endoplasmic reticulum were dilated, however, hepatocytes of test group were not damaged.

Saponins in nature are terpenoides with side chain and they occur naturally as glycosides in plants. These glycosides (saponins) were long known to lower the surface tension of water and therefore their aqueous solution froth readily and saponins cause hemolysis. It is easily understood from their structure that they are amphiphatic having both hydrophobic sapogenin aglycon part and hydrophilic sugar moiety in the molecule, and therefore, they disperse lipids in aqueous medium. The studies of saponins from various sources, however, show that they behave differently from each other. Some are toxic but the others are not: some are hemolytic while the others are protective to hemolysis.[1] It was realized that HD50 of the saponin fraction extracted from *Panax ginseng* C.A. Meyer was over 10^{-3}%.[2]

We examined the effect of either purified ginsenoside or the saponin fraction of *Panax ginseng* C.A. Meyer on various enzymes such as dehydrogenases, transaminases, lipase and found that moderate amount (10^{-6}%~10^{-4}%) of the saponin stimulated the all enzyme catalyzed reaction so far tested *in vitro* but their higher concentration inhibited the enzyme reactions unexceptionally (Fig. 1).

The Michaelis constants (Km) of various enzymes for their substrates were lowered in the presence of moderate amounts of the saponins. UV difference

	Concentration of ginseng saponin in assay mixture							
	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}	1 (%)
SDH (chicken liver)			↑		↓			
MDH (chicken liver)		↑			↓			
α-KGDH (chicken liver)					↑			
L-GLDH (rat liver)			↑			↓		
TP (rat liver)					↑			
AP (rat liver)				↑				
GOT (human serum)			↑	↑				
GPT (human serum)				↑				
ALDH (rat liver)	↑			↓				
ADH (rat liver)	↑		↓					
LPL (rat pancreas)				↑		↓		
G6PDH (human blood cell)			↑	↓				
MOase (rat liver)		↑						
MEOS (rat liver)		↑						
	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}	1(%)

Fig. 1. The effect of ginseng saponin on enzyme calalyzed reactions. The corresponding enzyme reaction rate reached maximum at the concentrations shown by mark (↑) and inhibitied when the saponin concentration was over those shown by mark (↓).
Abbreviation: Succinate dehydrogenase (SDH), malate dehydrogenase (MDH), α-Keto-glutarate dehydrogenase (α-KGDH), Isocitrate dehydrogenase (ICDH), L-Glutamate dehydrogenase (GLDH), Glutamate-oxaloacetate transaminase (GOT), Glutamate-pyruvate dehydrogenase (GPT), Aldehyde dehydrogenase (ALDH), Alcohol dehydrogenase (ADH), Lipoprotein lipase (LPL), Alkaline phosphatase (AP), Tryptophan pyrolase (TP), Glucose 6-phosphate dehydrogenase (G6PDH), Monoamine oxidase (MOase), Microsomal ethanol oxidizing system (MEOS).

spectra, CD spectra, electrophoretic mobilities, DTNB titration and substrate binding data demonstrated that moderate amounts of the saponins might bring about a slight change of the enzyme conformation which would be in favour for the enzyme reaction being proceeded. Other amphiphiles such as Triton X-100, and deoxycholate showed similar behavior as ginseng saponins do. The effects of several amphilpiles and ginseng saponin on several enzymes such as porcine pancreatic lipase and succinate dehydrogenase were examined and found that the optimal concentration of the amphilphiles for the maximum enzyme acitivity were found to almost the same range (10^{-4}%~10^{-3}%).[3-5] Therefore we have considered that the biphasic and nonspecific action of the saponins might be due to their surface acitivity and suggested that the surface activity of the saponin might play a significant role on the enzyme catalyzed reaction.[6,7]

Our ginseng saponin absorption experiment in rats using ^{14}C-labelled saponing from ^{14}C-acetate using ginseng root slices as enzyme source showed that ginsenosides were absorbed partly in the undissociated form and the saponin level in the liver might be maintained at 10^{-6}%~10^{-5}% in ginseng administered rats. The turnover rate of the saponins was relatively fast and half life of ginsenoside Rb_1 was estimated to be about 5 hours.[8,9] From the above considerations, it can be expected that ginseng saponins might stimulate unfavoured metabolisms and/or detoxication of toxic substances by raising up the related enzyme activities *in vivo*.

Ethanol is one of favorite mood-altering drug and its psychic effects, both pleasant and unpleasant, are well known enough but what is less known is that alcohol is a toxic drug; its overconsumption taxes the body's economy, produced a number of pathological changes, particularly in the liver and impairs biological functions.

Acetaldehyde is produced during ethanol meta-

Table 1. Subcellular distribution of aldehyde dehydrogenase (ALDH) in rat liver

Subcellular fraction	Relative activity (%)
Mitochondria	51.2
Cytosol	17.3
Microsome	34.0

bolism and is known to be oxidized mainly by aldehyde dehydrogenase (ALDH). Table 1 showed that the ALDH acitivty of rat liver was found mainly in the mitochondrial fraction but a significant ALDH activity was also present in microsomal fraction and cytosol fraction.[10] As shown in Table 2, there were optimum concentrations of the ginseng saponins for the maximum acitivity of enzymes such as ADH, ALDH, MEOS, respectively.

Wistar rats (150~200g, male) were given freely with 12% ethanol (Control) and/or 12% ethanol containing 0.1% ginseng saponins (Test) instead of water for 6 days and the liver was analyzed. Liver homogenate was used for ADH, ALDH, and MEOS activity analysis. As shown in Table 3, ALDH activities of both control and test group were lower than that of normal group but test ALDH was much less inhibited than control. ADH activities of both control and test were slightly higher than that of normal group. We know that ADH activity is usually stimulated by ethanol feeding at initial stage but our previous data showed that it became gradually steady after a prolonged ethanol feeding. MEOS activities of both control and test group were much higher than that of normal group. MEOS enzymes are inducible but the activity of test group was greatly higher than that of control.

Table 3. The effect of ginseng saponin on alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH), and microsomal ethanol oxidizing system (MEOS) in prolonged ethanol fed rats *in vivo*. The rats were fed with 10% ethanol (control) and/or 10% ethanol containing 0.1% ginseng saponin (test) instead of water 6 days

Group	ADH (unit[a]/mg protein)	ALDH ((unit[a]/mg protein)	MEOS (unit[b]/mg protein
Normal	8.743+0.159 (100)	3.076+0.600 (100)	3.165+0.472 (100)
Control	10.136+0.221 (116)	2.303+0.661 (75)	4.443+0.681 (140)
Test	9.242+0.123 (106)	2.678+0.015 (87)	7.028+0.775 (222)

[a] One unit was defined as the amount of enzyme to produce one nmole of NADH per min.

[b] One unit was defined as the amount of enzyme system to increase 0.01 of optical density at 420 nm per min.

Ethanol containing [1-^{14}C] ethanol (5 μCi) was

Table 2. The effect of the saponin fraction of *Panax ginseng* C.A. Meyer on alcohol dehydrogenase (ADH), cytosolic, mitochondrial and microsomal aldehyde dehydrogenase (ADLH) and microsomal ethanol oxidizing system (MEOS) of rat liver. Relative activity (mean of three determinations) was expressed assuming that of control being 100

Saponin conc. (%)	ADH	Mitochondrial ALDH	Cytoplasmic ALDH	Microsomal ALDH	MEOS
10^{10}	–	–	–	109	104
10^{9}	107	–	–	122	114
10^{8}	101	108	142	109	113
10^{7}	113	123	166	104	116
10^{6}	103	113	166	105	140
10^{5}	93	–	–	106	138
10^{4}	89	–	–	94	102
10^{3}	–	–	–	98	112

Table 4. Distribution of radioactivity (DPM) of hepatic lipids of rat which received intrapertoneal injection of 1 m*l* of 10% ethanol (containing [1-^{14}C] ethanol, 5Ci). The rats were killed 30 min later. Rats were fed with 12% ethanol (control) or 12% ethanol containing 0.1% saponin (test) instead of water for 6 days prior to [1-^{14}C] ethanol injection

Lipid fraction	Radioactivity (DPM)			Relative	Ratio
	Normal	Control	Test	C/N	T/N
Total lipid	114,089(100)	176,867(100)	142,637(100)	155.0	125.0
Phospholipid fraction	49,045(43.0)	12,407(7.0)	34,322(24.1)	25.3	70.0
Cholesterol fraction	10,528(9.2)	10,248(5.8)	10,267(7.2)	97.3	97.5
Fatty acid fraction	22,895(20.1)	49,820(28.2)	47,119(33.0)	217.6	205.8
Triglyceride fraction	835,817(31.4)	73,141(41.4)	59,326(41.6)	204.2	165.6

Table 5. Acetaldehyde level in the liver and serum of the rat fed with 12% ethanol and 0.1% ginseng saponin for 6 days prior to the intraperitoneal injection of 10% ethanol (1 m*l*). The rats were killed at 30 min after the ethanol injection

Group	Liver (nmole/g liver)	Serum (nmole/m*l* serum)
Normal	210.669± 98.611 (100)	12.139± 3.540 (100)
Ethanol fed	304.703± 119.506 (145)	17.594± 3.521 (145)
Ethanol and Saponin fed	238.343± 24.540 (113)	13.297± 2.512 (110)

* Numbers in brackets are the relative ratios that were expressed assuming that of normal being 100.

injected the above three groups. 30 min later, the distribution of radioactivity of hepatic lipids was investigatd. As shown in Table 4, radioactivities of hepatic lipids of both control and test group were higher than that of normal group, but that of test group was much lower than that of control.

Analysis of individual lipids such as phospholipids, cholesterol, fatty acid and triglycerides showed that phospholipid biosynthesis was significantly impaired and the synthesis of fatty acid and triglyceride was greatly stimulated. However, the saponin prevented the phospholipid biosynthesis depression and the increase of triglyceride biosynthesis considerably. It seemed that the ginseng saponin might stimulate ADH, ALDH and MEOS and the removal of acetaldehyde formed was acceleraled, and excess hydrogen can be shunt more quickly into lipid biosynthesis.

We analyzed acetaldehyde level in the liver and serum of rats fed with 12% ethanol and 0.1% ginseng saponin for 6 days prior to intraperitoneal injection of 10% ethanol (1 m*l*) at 30 min after the ethanol injection. As shown in Table 5, acetaldehyde level of saponin fed group was much lower than saponin non-fed group suggesting that the saponin might protect ALDH activity under some unfavorable conditions such as a heavy drink of etha-

Table 6. ALDH activity of the brain homogenate of rats which were free access to 12% ethanol instead of water for 5 days

Substrate	Acetaldehyde (4 mM)		Indole-3-acetaldehyde (3 mM)	
ALDH activity Group	Total acitivity (unit*)	Specific activity (nmole/min/mg protein)	Total activity (unit*)	Specific acitivity (nmole/min/mg protein)
Control	517.9(100)	2.4(100)	2596.1(100)	12.0(100)
Ethanol	352.7(68.1)	1.7(69.6)	2039.1(78.5)	9.7(80.3)

* One unit was defined as the amount of enzyme to produce 1 nmole per min under the experimental conditions. The figures in blacket are relative % assuming that of control being 100.

Table 7. Distribution of aldehyde dehydrogenase in rat brain

Fraction	Relative activity (%)
Cytosol	21.8
Mitocondria	58.4
Microsome	19.8

Relative activities are expressed as percent of the total activity found in the corresponding fractions.
(Assayed with 600 μM *p*-nitrobenzaldehyde as substrate).

Table 8. Distribution of aldehyde reductase in rat brain

Fraction	Relative activity (%)
Cytosol	96.1
Mitocondria	N.D.
Microsome	1 3.9

N.D.: not detected
Relative activities are expressed as percent of the total activity found in the corrdsponding fractions.
(Assated with 600 μM *p*-nitrobenzaldehyde as substrate).

Table 9. Effect of ginseng saponin on rat brain mitochondrial aldehyde dehydrogenase

Saponin (%)	Activity (unit*)	Relative activity (%)
0	8.79± 0.076	100.0
10^{-11}	8.74± 0.152	99.4
10^{-10}	9.43± 0.201**	107.3
10^{-9}	9.49± 0.263***	107.9
10^{-8}	9.16± 0.347	104.3
10^{-7}	9.06± 0.330	103.0
10^{-6}	8.95± 0.076	101.8
10^{-5}	8.31± 0.273	94.5

* One unit of enzyme was defined as nmole of NADH produced per min.
**p<0.01
***p<0.0005

Table 10. Effect of ginseng saponin on rat brain cytosolic aldehyde reductase

Saponin (%)	Activity (unit*)	Relative activity (%)
0	79.72± 2.463	100.0
10^{-11}	84.00± 0.328**	105.4
10^{-10}	87.75± 0.379***	110.1
10^{-9}	88.42± 3.938**	110.9
10^{-8}	91.64± 1.807****	115.0
10^{-7}	95.52± 1.366***	119.8
10^{-6}	86.28± 4.480	108.2
10^{-5}	91.10± 2.185	114.3

* One unit of enzyme was defined as nmole of NADH produced per min.
**<0.05
***p<0.01
****p<0.0005

Table 11. Effect of acetaldehyde on rat brain mitochondrial aldehyde dehydrogenase

Acetaldehyde (mM)	Activity (unit*)	Relative activity (%)
Control	13.40± 0.24	100.0
0.2	13.02± 0.23	97.2
2.0	12.97± 0.33	96.8
4.0	12.54± 0.35**	93.6
20.0	11.41± 0.47***	85.2

* One unit of the enzyme activity was defined as one nmole of NADH produced per min under the condition described in the text (600 μM of *p*-nitrobenzaldehyde was used as substrate in the reaction mixture).
**p<0.025
***p<0.005

nol.

When a large amount of ethanol was fed, a portion of acetaldehyde formed during ethanol oxidation in the liver will be transported through blood vessel to other organs such as brain and kidney. Accordingly, acetaldehyde level in brain will be raised but its high level of acetaldehyde is known to inhibit brain ALDH activity. The function of brain ALDH is now known to concern mainly with the oxidation of biogenic aldehydes which is derived from neurotransmitters such as indolamine and serotonin by the action of monoamine oxidase in brain. Therefore, when brain ALDH was inhibited by acetaldehyde, it is easily expected to occur some metabolic trouble. $NADP^+$ dependent aldehyde reductase (ALR) is also known to reduce some biogenic aldehydes to protect biogenic aldehyde accumulation in brain cells.

We analyzed the ALDH activity of brain homoge-

Table 12. Effect of acetaldehyde on rat brain cytosolic aldehyde reductase

Acetaldehyde (mM)	Activity (unit*)	Relative activity (%)
Control	48.50	100.0
0.2	47.43	97.8
2.0	47.34	97.6
20.0	47.34	97.6

* One unit of the enzyme activity was defined as one nmole of NADH produced per min under the condition described in the text (600 μM of *p*-nitrobenzaldehyde was used as substrate in the reaction mixture).

Table 13. Effect of ethanol on rat brain cytosolic aldehyde reductase

Ethanol concentration (mM)	Activity (unit*)	Relative activity (%)
Control	88.15± 2.650	100.0
0.25	83.47± 0.189**	94.7
2.5	83.20± 0.868**	94.4
25	79.31± 0.501***	90.0
50	77.57± 0.568***	88.0
100	73.29± 1.055****	82.4

*One unt of enzyme was defined as nmole of NADPH decreased per min.
**$p<0.05$
***$p<0.01$
****$p<0.005$

nate of rats fed with 12% ethanol instead of water for 5 days and found that ALDH activity of ethanol fed group was much lower than that of non-ethanol fed group, suggesting that the acetaldehyde formed from ethanol might be one of the reasons of some metabnolic disorders in brain (Table 6).

Distribution of ALDH (Table 7) and ALR (Table 8) activities in rat brain was also investigated and found that most of ALDH activities were found in mitochondria but a activity was in both cytosol and microsomal fractions. However, ALR activity was only in cytosol fraction. We realized that both ALDH and ALR were stimulated by ginseng saponin when the final concentration of the saponin in a reaction mixture was around 10^{-9}% (Table 9 and 10). Acetaldehyde (4 mM) inhibited rat brain mitochondrial ALDH (Table 11) but not cytosolic ALR (Table 12). However, ethanol (0.25 mM) was not good for ALR activity (Table 13).

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