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Effect of Chronic Treatment of Ginseng Extract on the Clearance of Blood Carbon Monoxide in Rat

Young-Gu Lee, Hyung-Ok Sohn, Heung-Bin Lim and Dong-Wook Lee*
Laboratory of Biochemistry, Korea Ginseng and Tobacco Research Institute, Taejon 305-345, Korea
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Abstract□The effect of long-term ginseng (*Panax ginseng* C.A. Meyer) administration on the clearance of carboxyhemoglobin (CO-Hb) and the property of blood gases was investigated in rats. Rats were received ginseng water extract (0.025% in drinking water) for 42 weeks starting at the age of 6 weeks. They were exposed to the diluted mainstream smoke generated from 15 filter cigarettes for 20 min in a round polycarbonate chamber (D37 cm×H13 cm). Under this condition, the mean CO-Hb content of control and the ginseng-treated rats immediately after the exposure was nearly the same as 13.8± 2.9 % and 13.9± 1.6%, respectively. However, CO-Hb was more rapidly removed from blood in the ginseng-treated rats than in untreated control with the laps of time, namely, its biological half life in the former was 36.9± 1.5 min and in the latter was 56.9± 13.2 min. Although long-term ginseng treatment did not affect the content of hemoglobin and blood pH of rats, it slightly increased blood oxygen content and its partial pressure value, and decreased levels of carbon dioxide and bicarbonate. These results suggest that long-term administration of rats with ginseng extract accelerate the elimination of CO from the blood. This effect seems to be related to the enhancement of oxygen consumption of the rat by a certain action of ginseng components as previously reported.

Key words□Ginseng, carboxyhemoglobin, carbon monoxide, cigarette smoke.

Introduction

Most living organisms require oxygen to survive. Oxygen is rapidly trnasported to tissues by hemoglobin. Adult human needs about 600 liters of oxygen per day and most of it is excreted from body as carbon dioxide.[1] Inhaled carbon monoxide (CO) is also readily absorbed into the blood stream. The CO binds to hemoglobin with the affinity 250 times more than the oxygen forming CO-hemoglobin complex.[2] When organisms are exposed to a CO rich condition, they fall into the state of the internal asphyxiation resulting from the reduced oxygen carrying capacity of blood. This impaired aerobic metabolism caused the damage of cells, tissues and organs.[3,4] The CO exposure causes the functional defect of brain,[5,6] and the increase of blood sugar level due to the reduced glycogen synthesis in the liver.[7] CO is one of undesirable components exist in the cigarette smoke and in the fire atmospheres.[8]

Ginseng (*Panax ginseng* C.A. Meyer) has been utilized for thousands of years as a mysterious herbal medicine, and various efficacies were observed in both of human and animals.[9-14] Especially, ginseng revealed to enhance the oxygen consumption of tissues and to improve the function of the damaged brain.[15,16] Ginseng and some other medicinal plants have a common characteristics, namely, their beneficial effects are not limited to a certain specific organ and were more effective in the organisms when were treated with them for long-term.[17,18] A typical efficacy of ginseng is to enhance the vitality of organisms, which are represented in various indices without any apparent side effect.[19]

These facts suggest that ginseng may be contributed to the improvement of the function of circulation system. And our preliminary study suggested the possibility that long-term administration of gin-

*To whom correspondence should be addressed.

seng would enhance the elimination of CO from the peripheral blood.

In order to manifest this hypothesis, we administered orally ginseng water extract to rats from 6 weeks after birth for 42 weeks and measured the elimination rate of CO-Hb from the blood of rats exposed to cigarette smoke.

Materials and Methods

1. Reagents

Sodium hydrosulfite, toluene and ammonium hydroxide were obtained from Aldrich Chemical Co. Other chemicals were of analytical purity grade. Oxygen gas (purity : up to 99%) used was purchased from commercial source.

2. Preparation of ginseng extract

Ginseng (roots of 6 years old) produced by Korea Tobacco and Ginseng Corporation was used. The extract was made from ginseng powder (30~40 mesh) by soaking it for 4 hrs in 5 volumes of hot water. Temperature of the water was maintained at 70℃ to prevent saponins and other phenolic compounds from being destroyed by heat. This procedure was repeated more than twice and the extract was pooled. The extract concentrated by using a evaporator was characterized by HPLC, and contained 36% moisture and 7% saponins. The major saponins present in the extract were composed of ginsenoside-Ro (0.09%), -Ra (0.06%), -Rb_1 (0.32%), -Rb_2 (0.01%), -Rc (0.18%), -Rd (0.22%), -Re (0.08%), -Rf (0.05%), -Rg_1 (0.15%) and -Rg_2 (0.82%).

3. Animals

Male Sprague-Dawley rats of age 6 weeks divided into two groups: 8 rats each for control and ginseng treated groups. All rats were housed in a clean conventional barrier system under the condition of a 12 hrs light/dark cycle at 200~300 lux, 22± 2℃ in temperature and 40~60% in relative humidity. The ginseng-treated rats were received ginseng water extract (0.025% in drinking water) for 42 weeks. The control group received only water. Water and the ginseng solution were given and replaced with a fresh ones every days.

4. Cigarette smoke inhalation

Rats of 12 months of age from control and the ginseng-treated groups were exposed to the diluted mainstream smoke (1 : 8, smoke/air) in a round polycarbonate chamber. The smoke generated from 15 cigarettes (content) of total particulate matter and nicotine was 11 and 1.1 mg/cigarette, respectively) was supplied continuously into the chamber for 20 min by automatic smoking machine (Heiner Borgwaldt II). The duration of each puff was 2 sec and the measured volume was 35 m*l*. A round cylindrical polycarbonate chamber contained 8 separate compartment, each housing one rat, was used.

5. Determination of carboxyhemoglobin

Blood was drawn from the rats at 0, 30, 60, and 120 min after cigarette smoke exposure. The content of carboxyhemoglobin (CO-Hb) was determined by the method of Watson *et al.*[20] Briefly, blood (10 μ*l*) was diluted in 20 m*l* of 0.04% ammonia. The diluted blood sample was divided into two portions and one portion was oxygenated by bubbling pure oxygen through the solution for 20 min, and the absorbance of the oxygenated sample was measured at 540 and 500 nm against 0.04% ammonia solution as a blank. The difference spectrum (d) of CO-Hb saturated portion of the sample was recorded against its corresponding oxygenated samples and optical peak height (ho) value for the instrument was determined from calibrationn curve made from the observed d value. Remaining half was then placed in the sample cuvette and read the absorbance against oxygenated sample at 405, 438 and 420 nm. The height (h) was calculated by A420- (A438-A405) /2. CO-Hb concentration of the sample was determined according to the formula: % CO-Hb=h/ho× 100.

6. Blood gas analysis

The measurement of partial pressure of oxygen and CO, and other parameters of blood was performed using a blood gas analyzer (NOVA Biomedical, No STAT-Profile 3, USA).

Biological half life of CO-Hb was calculated from values of the log concentration of CO-Hb vs. time curve after the exposure of rats to cigarette smoke. Data were analyzed statistically using T-Test software.

Results and Discussion

The aim of the present study was to investigate the effect of ginseng on the elimination of CO from blood of rats imposed by cigarette smoke inhalation. Fig. 1 shows the change in body weight of rats during the treatment. The body weight gain was slightly higher in the ginseng-treated rats than in control ones, however, there was no significant difference between two groups as previously reported.[17,18] The result suggests that chronic ginseng treatment do not affect the body weight and has an effect to enhance the vitality of rats.

As a preliminary experiment, we observed the change of CO-Hb content with the exposure time in another group of control rats. The CO-Hb shows a typical absorption spectrum with a peak at 420 nm and a trough at 405 nm (Fig. 2) and there was a positive linear relationship between the exposure time and the resultant percent of CO-Hb saturation. Namely, unexposed rats contained less than 0.8% of CO-Hb, but the exposure of rats to the cigarette smoke for 10 and 20 min under the present experimental condition marked 9.1± 1.6 and 13.8± 2.2% of CO-Hb, respectively (Table 1).

In human, CO exposure is known to have cardiovascular, neurobehavioural and fibrinolytic effect.[21] CO-Hb content of rural and urban people is commonly 0.4~0.7% and 1.2%, respectively. And that of current smokers is about 5~6%.[22] This concentration of CO-Hb does not induce health problems.[21] However, the existence of 7~20% of CO in the air leads to statistically significant decrease in maximum oxygen consumption during strenuous exercise in young healthy man.[22] Based on the literatures, we extablished the condition of 20 min exposure in investigating the ginseng effect on CO-Hb elimination from blood.

Fig. 3 shows the effect of ginseng treatment on CO elimination from the blood. The basal content of CO-Hb in both groups of rats without smoke exposure was about 0.7%. Immediately after the exposure, the content of CO-Hb was 13.8± 2.9% in control and 13.9± 1.6% in ginseng-treated rats, respectively showing no effect. However, it was significantly lower in the ginseng-treated rats (7.5± 1.1%) than in control ones (10.4± 0.9%) at 30 min after the exposure. Such a trend was maintained until

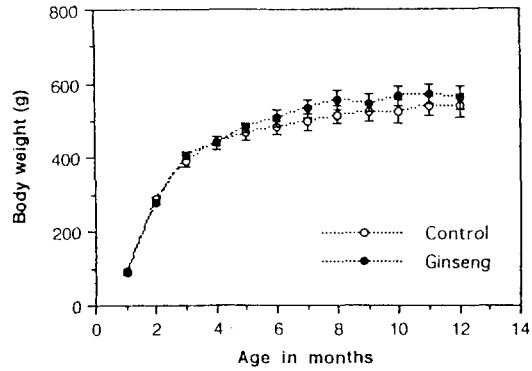

Fig. 1. Effect of long-term administration of ginseng on body weight gain of rats.

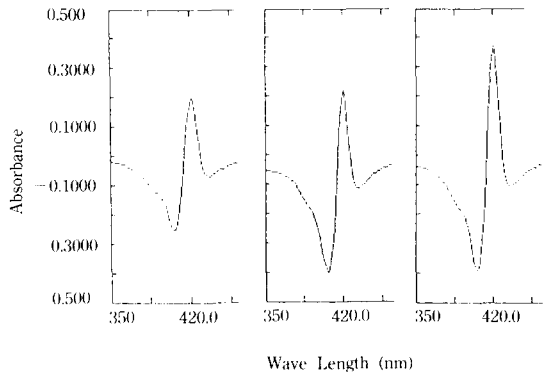

Fig. 2. A typical absorption spectra of CO-Hb. The difference of optical density between corresponding CO-Hb and oxygenated hemoglobin. (A : 5 μl, B : 10 μl and C : 15 μl of blood samples).

60 mins later, but the content fell to around 3% after 2 hr in the both groups. The half life of CO-Hb in control rats was 56.9± 13.2 min in similar to the previous value (Fig. 4). But, it decreased to 36.9± 1.5 mins in the ginseng-treated rats suggesting the accelerated clearance of CO-Hb from blood by ginseng. The half life of CO-Hb in blood varies according to the species from 30 mins in mice to 4.5 hrs in human.[20] That of conrol rats observed in this study exhibited a similar value to the previous results.

This is the first report showing beneficial efficacy of ginseng on CO elimination from the blood. Several possibilities could be thought about the mechanism under the ginseng effect on CO elimination. As the best possible explanation, ginseng may inc-

Table 1. Relative concentration of CO-Hb in rat blood with the duration of cigarette smoke exposure

Duration of smoke exposure (mins)	CO-Hb concentration (%)
0(unexposed)	0.8± 0.4
10	9.1± 1.6
20	13.8± 2.2
30	17.1± 2.5

Rats were exposed to cigarette smoke with continuous flew from machine as described in "Materials and Methods". Cigarettes were replaced with new ones at every 10 mins (data were expressed as mean± SD, n= 8).

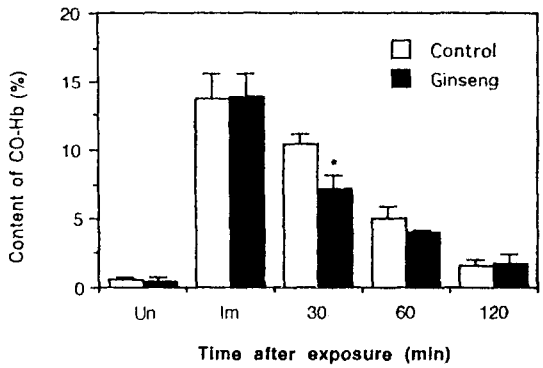

Fig. 3. The content of CO-Hb in blood of rats after exposure to the cigarette smoke. Details are described in "Materials and Methods" (Un : Unexposed to the smoke, Im : Immediately after exposure). *Significantly different from control ($p<0.05$).

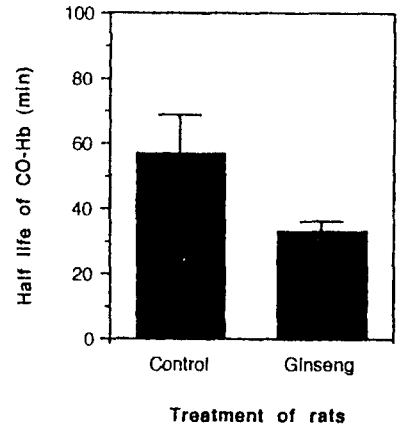

Fig. 4. The biological half life of CO-Hb in the blood of control and ginseng treated rats.

Table 2. The effect of ginseng treatment on the property of blood gases in rats

Parameters	Control	Ginseng
pH	7.46± 0.02	7.40± 0.08
Hb (mg/dl)	14.3	14.3
O_2Ct (ml/dl)	18.2± 0.3	19.0± 0.5
P_{O_2} (mmHg)	75.5± 0.3	80.5± 7.2
P_{CO_2} (mmHg)	37.0± 3.7	36.6± 2.2
TCO_2 (mmole/l)	27.3± 2.3	24.4± 3.8
HCO_3 (mmole/l)	26.2± 2.2	23.3± 3.8

Blood obtained from tail vein of rats prior cigarette smoke exposure was used for the analysis of these parameters. O_2Ct : Oxygen content (Data were expressed as mean± SD).

rease the partial pressure of oxygen in peripheral blood because it is the most important factor changeable to affinity of hemoglobin to oxygen. Von Ardenne and Klemm[24] demonstrated that chronic treatment of human with 200 mg of ginseng extract per day for 4 weeks causes to increase oxygen consumption of tissues. Such an effect of ginseng was also observed an *in vitro* experiment by Aprikian and his colleagues.[25] These findings suggest that ginseng enhance oxygen consumption of tissues by a certain mechanism. As a consequence of such an action, organism might be maintained high oxygen partial pressure in blood. To validate this hypothesis, we compared the partial pressure and content of oxygen and carbon dioxide in the blood of control and ginseng-treated rats. As shown in Table 2, the content of oxygen and its partial pressure value were slightly higher in the rats treated ginseng chronically than in control ones. On the contrary, partial pressure of carbon dioxide and contents of carbon dioxide and bicarbonate were relatively low in the ginseng-treated rats although there was no significant difference. Other parameters such as hemoglobin and blood pH were, however, not changed by long-term treatment of ginseng. Therefore, a slight change in the property of blood gases seems to be related to the acceleration of CO-Hb elimination.

Several other possibilities can be also considered in connection with ginseng effect on CO elimination. One of them is the influence of ginseng on

the other effecting factors on hemoglobin affinity to oxygen. The affinity of hemoglobin is known to be affected by various factors such as pH, body temperature, carbon dioxide, ATP or 2,3-diphosphoglycerate besides the partial pressure of oxygen. Since such parameters are closely interrelated, the alteration in any one would influence the oxygen affinity.[26] The ginseng effect on the body temperature would be an evidence, which is apparent in the subpopulation with a certain physical constitution. Second possibility is the control of respiratory center of brain by the treatment of ginseng, for ginseng stimulates the release of neurotransmitters such as dopamine, norepinephrine and epinephrine in striatal region of brain[27], which are known to be involved in the contol of respiratory center.

Antioxidant action of ginseng[14, 17, 18, 28] could be also a candidator on this positive aspect, because iron atom in hemoglobin does not react with oxygen althought it will bind certain ligands such as cyanide when it is oxidized to the trivalent state. Therefore, antioxidant action by a certain components or pharmacological roles of ginseng might be contributed to maintain the iron of hemoglobin in the divalent state. However, in the present study, we did not provide any clear evidences for the above other possiblities althogh ginseng has showed an obvious effect to accelerate the CO elimination in rat blood. Further study is necessary to elucidate its mechanism.

The oxygen theraphy is a popular treatment for patients of CO poisoning, which also reduce the blood CO-Hb concentration.[29] However, it requires an extra caution because the hyperoxia can induce the free radical toxicity *in vivo*.[30, 31]

In conculsion, the chronic ginseng treatment demonstrated the positive effect of the increased elimination of CO-Hb, which could be beneficial to the smokers.

요 약

인삼의 장기적인 급여가 흰쥐의 혈중 CO-Hb의 제거에 미치는 영향을 조사하였다. 인삼의 물추출물을 음수에 타서(0.025%) 42주 동안 흰쥐에 투여하고, CO-Hb의 형성을 위해 이들을 15개피의 담배를 연소시켜 얻은 연기에 20분간 전신폭로법으로 노출시켰다. 이 조건에서 흡연직후 CO-Hb의 농도는 대조군이 13.8±2.9%이었고, 인삼투여군이 13.9±1.6%로 서로 비슷하였다. 그러나 CO-Hb의 생물학적 반감기는 인삼투여군에서는 36.9±1.5분인데 비해, 대조군에서는 56.9±13.2분으로 인삼투여군이 훨씬 빨랐다. 한편, 혈액의 pH나 hemoglobin은 인삼투여로 인해 변화되지 않았으나 혈액의 산소분압은 다소 증가되었고, 이산화탄소의 분압은 다소 감소되었다. 이 결과는 인삼의 장기적인 급여로 혈중 CO-Hb의 제거가 촉진된다는 것을 보여주며, 이것은 인삼이 산소소모를 촉진시키는것(Von Ardenne *et al.*, 1987; Aprikian *et al.*, 1993)과 관련이 있는 것 같다.

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