STUDIES OF GINSENG EXTRACT ON AGE-RELATED ENZYMES

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INTRODUCTION

All biological reactions in the body are catalyzed by enzymes. They are, therefore, essential for various function of the body. Hence changes in their properties during ageing may alter the functional ability of an organism. A considerable amount of data has accumulated which show that there are remarkable changes in specific activity, Km, heat stability, isozyme pattern and spectroscopic properties (1-24). It is not clear how they happen. But there are many suggestion for them; biosynthetic error, conformational changes by somatic mutation, post-translational modification and mixed oxidation system (26-31). Our team has been working on age related enzymes such as isocitrate dehydrogenase (E.C. 1.1.1.42), 6-phosphogluconate dehydrogenase (E.C. 1.1.1.44), glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49), glutathione peroxidase (E.C. 1.11.1.9) and glutathione reductase (E.C. 1.6.4.2), and seeking any possible candidate to maintain or retard decline of enzyme activity during ageing. Ginseng extract has been shown to enhance enzymatic activity *in virtro* by our team. Such results stimulate us to see if there are any changes in enzymatic proporties such as specific activity, heat stability, affinity for substrates, mobility in electrophoresis and immune response especially in glucose-6-phosphate dehydrogenase after oral adminstration of ginseng total extract on time course.

MATERIALS AND METHODS

1. Materials

6 year old Korean ginseng was grinded and extracted by using 70% EtOH 3 times (Ginseng extract). Extract was evaporated and lyophilized, and dissolved in distilled water for experiment (10mg/ml). After one month from birth, male Sprague-Dawley was given 10mg of extract/kg body weight orally every day for certain period ranging from 1/4 month to 15 month. Animal used for immune response of glucose-t-phosphate dehydrogenase was a white rabbit (New Zealand Strain, male). Chemicals for enzyme purification, and enzyme assay were purchased from Sigma Chemical Co., Chemicals for electrophoresis were obtained from Bio-Rad Co.. The rest of chemicals is of analytical grade.

2. Methods

Enzymatic activities such as isocitrate dehydrogenase, 6-phosphogluconate dehydrogenase, glucose-6-phosphate dehydrogenase, glutathione peroxidase and glutathione reductase were determined as described elsewhere (32-34) Heat stability of enzyme was carried out by rasing appropriate temperature for 5 minute intervals and cooling rapidly and followed by enzyme assay. Determination of Kim, electrophoresis were made by using method (35-37)

RESULTS AND DISCUSSION

Specific activities of glucose-6-phosphate dehydrogenase (G6PDH) and isocitrate dehydrogenase (ICDH) were observed at various ages (Figure 1 and 2). They reached maximum at age of 2 and 3 month, respectively and decreased steadily. Such trends are in a good agreement with previous results (26). However, specific activities of 6-phosphogluconate dehydrogenase (6PGDH), glutathione reductase (GR) and glutathione peroxidase(GP) were shown to have no maximal ages like G6PDH and ICDH (Figure are not presented). As shown Figure 1 and 2, both activities of G5PDH and ICDH were remarkably

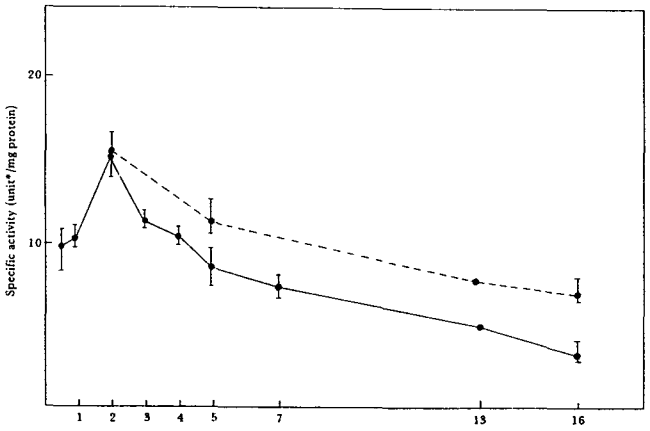

Fig. 1. Specific activity of liver glucose-6-phosphate dehydrogenase from rat administered with total ginseng total extract and without on time course. ●——●, without ginseng total extract; ●----●, with ginseng total extract. one unit: In mole NADPH formed/min.

different between 2 month or 3 month and 16 month old rat adminstered with ginseng extract. Such enhancement of enzyme activity by ginseng extract was also observed in 6PGDH, GR, and GP as summerized in Table 1. GP activity was most significantly enhanced. But there were slight enhancement of enzyme activity such as G6PDH, 6PGDH and ICDH in rats adminstered with ginseng extract for certain period ranging from 1 week to 4 weeks (data are not presented). Preliminary as results are, longer term adminstration of ginseng extract seems to be better than short term. Such enhancement of enzyme activity by

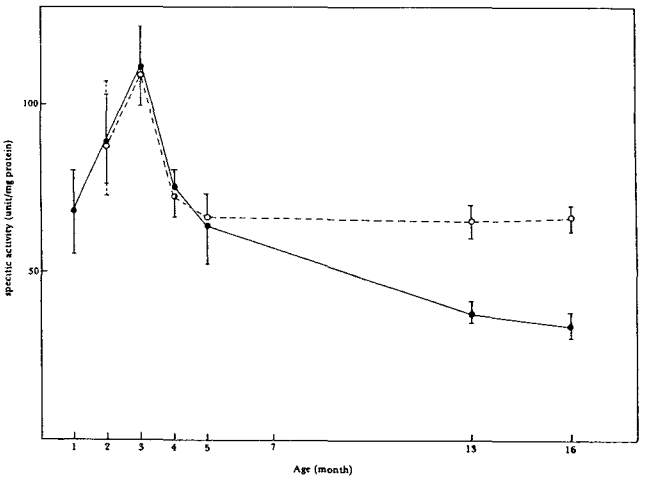

Fig. 2. Specific activity of liver isocitrate dehydrogenase from rat administered with ginseng extract and without on time course.
○——○, without ginseng extract; ○-----○, with ginseng extract.
* one unit: 1 nmole NADPH formed/min

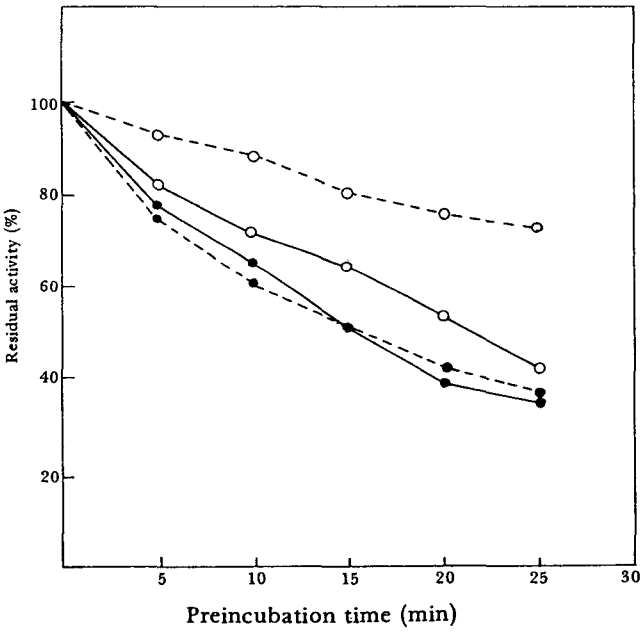

Fig. 3. Heat stability of liver glucose-6-phosphate dehydrogenase from 2 month and 16 month old rats administered with ginseng total extract and without. (43°C)
●——●, 2 month old; ●----●, 2 month old + ginseng total extract; ○——○, 16 month old; ○-----○, 16 month old + ginseng total extract.

ginseng extract could possibly prevent accumulation of toxic peroxides since higher production of NADPH by G6PDH, 6PGDH and ICDH can be

Table 1. Specific activities of age-related enzymes after adminstration of ginseng extract for 1 and 15 month.

Enzymes	Age	Specific activity (unit/mg proter)	Relative activity A/NA	Relative activity 16month/2month
G6PDH	2 NA	15.53 ± 1.43	100	100
	A	16.28 ± 1.25	105 ± 7.68	
	16 NA	3.17 ± 0.067	100	21
	A	6.73 ± 0.929	209 ± 13.80	43
6PGDH	2 NA	8.95 ± 0.15	100	100
	A	9.13 ± 0.64	102 ± 7.01	
	16 NA	4.70 ± 0.551	100	53
	A	7.30 ± 0.334	155 ± 4.58	82
ICDH	2 NA	89.86 ± 13.07	100	100
	A	88.00 ± 14.32	98 ± 16.27	
	16 NA	34.80 ± 4.41	100	39
	A	67.86 ± 3.65	195 ± 5.38	76
Glutathione reductase	2 NA	13.0	100	100
	A	19.67 ± 0.667	151 ± 5.13	
	16 NA	6.3	100	49
	A	16.67 ± 2.73	265 ± 16.4	
Glutathione peroxidase	2 NA	99	100	100
	A	115 ± 15.99	116 ± 13.90	
	16 NA	19	100	19
	A	82.67 ± 17.65	435 ± 21.37	84

* Adminstration was made after one month from birth

* 100 was arbitrarily given to the control

utilized for formation of reduced glutathione by GR, which GP can use for following reaction.

$$2GSH + H_2O_2 = G\text{-}S\text{-}S\text{-}G + 2H_2O.$$

Therefore, 5 enzymes could partly retard accumulation of toxic peroxides during ageing. In addition to enhancement of enzyme activity by ginseng extract, the other properties such as heat stability, Km, electrophoresis pattern and immune response of G6PDH was observed at age of 2 month and 16 month since G6PDH has maximum specific activity at age of 2 month and lower specific activity at age of 16 month. As shown in Figure 3, G6PDH from rat adminstered with ginseng extract for 15 month was shown to have higher resistance to heat than anyother whereas that of rat for one month was shown to have similar behavior to the control. Km for glucose-6-phosphate was shown to reduce very significantly in case of adminstration of ginsec extract for 15 month as shown Figure 4. Km for $NADP^+$ was also shown to reduce significantly in rat adminstered with ginseng extract for 15 month.(Figure 5) Reduction of Km values for $NADP^+$ and glucose-6-phosphate suggests that ginseng extract could have an effect on their binding sites. Our team has observed enhancement of specific activity and reduction of Km values *in vitro* by ginseng extract. Therefore ginseng extract has an effect on enzyme *in vitro* and *in vivo* as far as certain enzymes are concerned. No distinct difference of isozyme pattern on electrophoresis by activity staining was observed among 2 month old and 16 month old rats, and

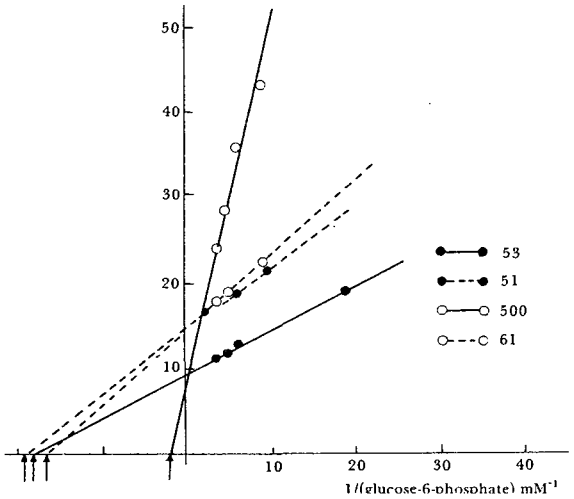

Fig. 4. Lineweaver-Burk plot for the determination of Km values for glucose-6-phosphate.
●—●, 2 month old; ○-----○, 2 month old + ginseng total extract; ○----○, 16 month old; ○—○, 16 month old + ginseng total extract.
* V; ΔA/min. unit: μM

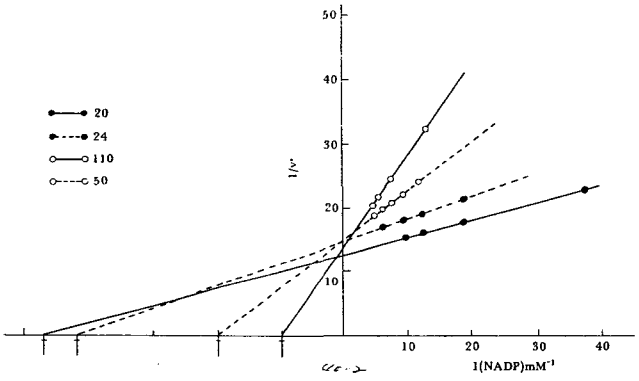

Fig. 5. Lineweaver-Burk plots for the determination of Km value for NADP.
●—●, 2 month old; ●-----●, 2 month old + ginseng total extract; ○—○, 16 month old; ○-----○, 16 month old + ginseng total extract.
* V; Δ A/min. unit: μM

both rats adminstered with ginseng extract for 1 month and 15 month, respectively. (data not presented) Such trends suggest that ginseng extract has no effect on isozyme pattern. No difference of immune response was also observed among them (Figure 6). Such results suggest that ginseng extract could have an effect on specific activity and Km values but ginseng extract have no effect on isozyme pattern.

Ginseng extract seems to enhance specific

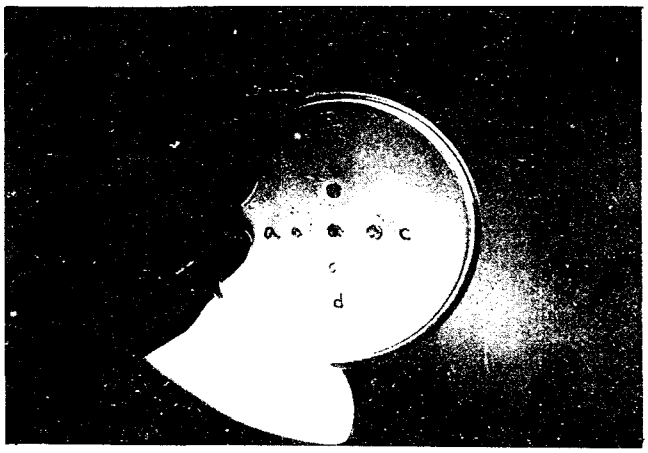

Fig. 6. Double-immunodiffusion of anti-glucose-6-phosphate dehydrogenase serum against the cell-free extracts on agar (1%, w/v) plate.
Ab, anti-G6PDH serum prepared from 2 month old rats; a, 2 month old; b, 2 month old + ginseng total extract;
c, 16 month old; d, 16 month old + ginseng total extract.

activity by reducing Km values through unknown mechanism which should have been clarified. Whereas ginseng extract seems to have no effect on electrophoresis pattern and immune response. Cumulative results, however, suggest that ginseng extract could retard decline of enzyme specific activity during ageing and produce more NADPH which can form more reduced glutathione, which could control hexose monophosphate shunt and prevent accumulation of toxic peroxides formed during ageing. Therefore ginseng extract could possibly retard senescence to some extent.

Cha: In your studies, have you kept feeding these rats for a long time to test for their life-time?

Cho: Do you mean more than 6 months?

Cha: Yes.

Cho: No, we didn't try yet but we are doing it now.

Cha: In the liver, reduced GSH level of 10mM, which is more than enough, much more than adequete. Normally, it is enough to take care of the life in the cell and the depletion of GSH is very quickly replenished from the mixed disulfide in the cell. Also, the turn-over rate of glutathione peroxidase is 100 times faster than the GSH reductase. From that point of

view your observation of increased glutathione peroxidase by 4 times is very significant. However, increase in GSH reductase and Glucose-6-P dehydrogenase as well as isocitrate dehydrogenase was also observed. I don't know whether they play a significant role in protecting *life* directly. I think that the most significant one in your observation is 4-times increase in GSH peroxidase.

Cho: It is very hard to answer because we didn't carry out in detail on that point of view.

K.S. Lee: May I ask you one more question in connection with the previous one? You measured the specific enzyme activity. What was the total enzyme activity per gm of tissue? Have you measured that?

Cho: Well, actually when we carried out the experiment, the isolated amount of liver tissue was different so we just calculated it on the basis of mg protein.

K.S. Lee: But you could calculate the total activity of different amount of tissue per gm of tissue.

Cho: Yes, we could but I didn't bring that data.

K.S. Lee: Do you mean that you have any data on that?

Cho: Yes, we have.

Tso: My slight concern is the enzyme activity of the liver. I'd like to know whether you found any difficulty in comparing the specific activity between the control and the treated one. Can you completely rule out the possibility of other protein biosynthesis? That means if you did go through all sorts of purification procedure, there is a slight possibility that it might not be the enzyme itself. That's why I'm asking you. Could you device some method that can compare the two enzymes.

Cho: As you have seen in the slide, the administration of ginseng extract didn't make any big difference in the protein as far as liver is concerned. We measured the RNAase activity, which means that if ginseng extract activate or inhibit in our *in-vivo* system, then the enzyme activity would be different. But there was no big difference between the administered one and non-administered one.

Tso: Since you also included GSH-peroxidase in your study, it seems to me that GSH-peroxidase is a very specific enzyme in the gonad as well, but it can be labelled with selenium in stead of sulfur. Then you can compare the control and treated one by determining the methionine incorporation.

Fulder: I think, however, some of the criticism of this study, which relate to the fact you made in the old animal, might not be the same as the young animal. In aging, I think, some enzyme activities go down and there also some enzyme activities increase. It does mean to say that old animals don't have to have low enzyme activity. I think, additional observation would have to be tested on one or two other enzymes which have increased activity with age. So, see if ginseng feeding also brings that back to the young level.

Cho: Thank you for your good suggestion. I didn't try yet the enzyme which increases when animals get older. As you mentioned, there are several enzymes whose activity increases with age.

고려인삼 추출물이 노화관련효소에 미치는 영향

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노화는 모든 다세포 유기물의 특징이다.

노화가 됨에 따라서 효소활성 및 면역반응의 감소와 과산화지질과 지방갈색물질의 축적, 효소와 염색질을 포함하는 단백질 구조의 변화, 호르몬계의 불균형 등이 일어난다. 그렇지만, 노화가 어떻게 일어나는지에 관하여는 현재까지 확실하지 않다.

본 연구진은 노화와 관련된 효소들에 관하여 연구를 하여 왔으며, 노화가 진행되는 동안의 효소의 활성을 유지시켜주거나, 또는 효소의 활성이 감소되는 것을 지연시켜 주는 물질을 찾고자 노력하였다.

그 가운데 하나로서, 고려인삼 추출물을 흰쥐에 기간별로 투여하여 효소활성의 차이, 열에 대한 안정성, 기질에 대한 친화력, 전기 영동 상의 이동성과 면역적인 반응을 대조군과 비교하였다.

Glucose-6-phosphate dehydrogenase, 6-phosphog-

luconate dehydrogenase, glutathione redutease, glutathion peroxidase와 같은 노화와 관련된 효소들의 활성을 고려인삼 추출물을 1개월간 흰쥐에(60~80g) 투여하여 대조군과 비교 조사하였으나, 별 차이가 없었다.

그러나 고려인삼 추출물을 15개월간 투여하였을 때에는 이러한 노화관련 효소들의 활성이 급격히 증가함이(70~200%) 관찰되었다.

예견된 바와 같이, 효소의 열에 대한 안정성과 기질에 대한 친화력도 증가함이 관찰되었다. 그러나 glucose-6-phosphate dehydrogenase의 경우에서 전기영동상의 차이 및 면역적인 반응은 대조군과 유사하였다.

이상의 결과는 고려인삼 추출물이 노화와 관련된 효소들의 활성이 감소되는 것을 지연시켜줄 수 있으며, 노화를 어느정도 지연시켜 줄 수 있음을 의미한다.

이와 같은 결과를 포함한 실험자료를 국제 인삼심포지움에서 발표할 것이다.

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