Vol. 126, No. 1, 1985 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 16, 1985 Pages 636-640

# GINSENG EXTRACT INHIBITS PROTEIN DEGRADATION AND STIMULATES PROTEIN SYNTHESIS IN HUMAN FIBROBLASTS

Zhong-Qi Lu[1] and J. Fred Dice[2]

Department of Physiology and Biophysics
Harvard Medical School, Boston, MA 02115

Received December 6, 1984

Summary: Aqueous extracts of Panax ginseng inhibit intracellular protein degradation in confluent cultures of IMR-90 human diploid fibroblasts. The magnitude of the inhibition is similar to that observed with insulin and polypeptide growth factors. Furthermore, the inhibition of proteolysis by ginseng, like that produced by insulin and growth factors, is selective in that it applies to long-lived proteins but not to short-lived proteins. Ginseng also stimulates protein synthesis in human fibroblasts indicating that components of ginseng extract are capable of acting directly on human cells to promote protein accumulation. 

Panax ginseng is one of the most widely used traditional Chinese medicines (1-4). It has been reported to alleviate symptoms of a variety of degenerative diseases including diabetes and old age (2,3). In several tissues of intact animals ginseng is known to have insulin-like effects including reduction of blood glucose (5-8) and serum triglyceride levels (9-11) and promotion of DNA, RNA, and protein synthesis (9-18). Certain studies have concluded that ginseng components stimulate insulin production by the pancreas (16) while others have claimed that ginseng extracts contain insulin-like substances capable of acting directly on cells (12-14).

One important action of insulin and polypeptide growth factors is to inhibit intracellular protein degradation (19,20,24). We now report that ginseng extracts inhibit proteolysis, as well as stimulating protein synthesis, in human fibroblasts.

## Materials and Methods

Cell Culture: The human embryonic lung fibroblast strain IMR-90 was obtained from the Institute for Medical Research (Camden, NJ) and grown to

[1] Permanent address: Department of Physiology, Capitol Medical College of China, Beijing, People's Republic of China

[2] To whom correspondence and reprint requests should be addressed

0006-291X/85 $1.50

confluency in DMEM[3] as described previously (20) except that 30 mm-diameter multiwell plates were used. Fibroblasts were used for experiments between population doubling levels of 20 and 30.

Ginseng Extract: Panax ginseng (C.A. Meyer) was purchased from China near Beijing. Water soluble extracts of the root were prepared as follows: Five grams of the root were minced and added to 50 ml of distilled water. The water was brought to a boil and was kept boiling for 30 min during which time approximately two-thirds of the water evaporated. The supernatant was decanted into a centrifuge tube and spun at 10,000 x g for 30 min. The final supernatant was sterilized by filtration through a 0.45 micron Millipore filter and stored at $4^{o}$C. until use. The concentration of the extract used in experiments is expressed as mg of the original root per ml. For example, if the recovery of supernatant after boiling was 20 ml, the concentration of original root per ml is taken to be 250 mg/ml. For most experiments this extract would then be diluted 28.7-fold to a final concentration of 8.7 mg/ml.

Protein Degradation: Rates of degradation of long-lived and short-lived proteins were determined simultaneously as described previously (20). In brief, confluent cultures were radiolabeled for 2 days with [$^{14}$C]leucine to label all cellular proteins, the majority of which are long-lived (20). Then the same cultures were exposed to [$^{3}$H]leucine for 1 hr to preferentially label short-lived proteins. The cultures were washed extensively, and medium containing 2.8 mM unlabeled leucine was added to minimize reutilization of the isotope (20). Samples of the medium were withdrawn at various times and the acid-soluble $^{3}$H and $^{14}$C radioactivity determined. Calculations of % radioactivity remaining in cells at individual time points were carried out as described (21).

Protein Synthesis: Rates of protein synthesis were determined as the incorporation of [$^{3}$H]leucine into acid-insoluble material in the presence of excess (2.8 mM) unlabled leucine in the medium. Under such "flooding" conditions incorporation of radioactivity into acid-insoluble material accurately reflects rates of protein synthesis (22) and minimizes differences due to alteration of amino acid transport and/or intracellular amino acid pool sizes (22).

## Results and Discussion

The effects of ginseng (8.7 mg/ml) and insulin ($10^{-6}$ M) on degradation of long-lived and short-lived cellular proteins are shown in Figure 1. Both ginseng (Figure 1a) and insulin (Figure 1b) inhibit the degradation of long-lived proteins by 30% - 40%. In the same cultures neither agent affects the degradation of short-lived proteins. These results were qualitatively similar for 3 different preparations of ginseng extract. Furthermore, the inhibition of proteolysis was rapidly reversible upon removal of the ginseng from the medium (data not shown).

Table 1 is a summary of experiments similar in design to those shown in Figure 1. Ginseng extract that was diluted two-fold had approximately one-half

[3] Abbreviations used: DMEM, Dulbecco's modified Eagle's medium

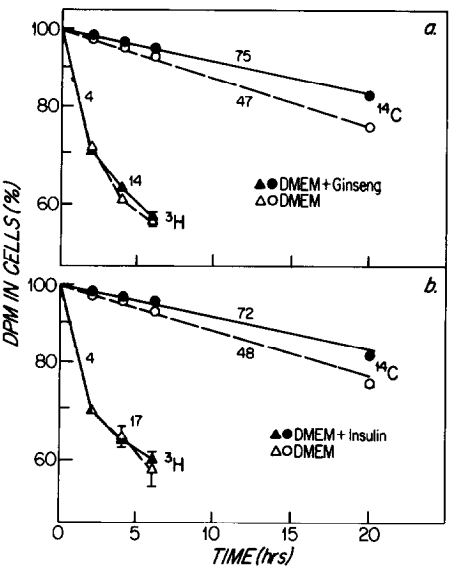


Figure 1: The Effect of Ginseng and Insulin on Protein Degradation.

Confluent monolayers of IMR-90 human fibroblasts were labeled with [$^{14}$C]leucine for 2 days and [$^{3}$H]leucine for 1 hr as described (20). The short pulse of $^{3}$H preferentially incorporates into into short-lived proteins while the $^{14}$C is more uniformly distributed throughout cell proteins most of which are long-lived (20). Samples of culture medium (DMEM ± additions) were taken at various times and acid-soluble radioactivity determined. Degradative curves were constructed as described (21), and the best fit line calculated by regression analysis. The results shown are averages ± one standard deviation for 3-5 wells. Where standard deviations are not shown, they fall within the boundries of the data points. The numbers on the figure indicate half-lives of the proteins. Analysis by a two-tailed Student's t-test indicated that both insulin and ginseng inhibited protein degradation at every time point ($.001 < p < .02$). Values for insulin and ginseng were not significantly different from each other at any time point ( $p > .05$)

of the inhibitory effect on catabolism of long-lived proteins. Unfortunately, attempts to analyze the effects of more concentrated extracts of ginseng were unsuccessful due to detachment of the cells from the culture dish. The toxicity of high concentrations of ginseng added to cultured cells has been previously noted by others (14). Addition of fetal bovine serum (10%), insulin ($10^{-6}$M), epidermal growth factor ($10^{-6}$M), or fibroblast growth factor ($10^{-6}$ M) also reduced degradation of long-lived proteins but not short-lived proteins. Other reports suggest that selective inhibition of degradation of long-lived proteins is a common response of cultured cells to growth factors (22-27). In contrast, lowered temperature (20,28) or metabolic poisons (25,29) reduce the degradation of both short-lived and long-lived proteins.

Table 1: Effects of Ginseng, Insulin, and Growth Factors on Protein Degradation in IMR-90 Human Fibroblasts

| Addition | Concentration | Inhibition of Degradation (%) | |
|---|---|---|---|
| | | Long-Lived Proteins | Short-Lived Proteins |
| ginseng | 8.7 mg/ml | 37 | 0 |
| | 4.35 mg/ml | 18 | 0 |
| *fetal bovine serum | 10 % | 29 | 0 |
| insulin | $10^{-6}$ M | 33 | 0 |
| *epidermal growth factor | $10^{-6}$ M | 31 | 0 |
| *fibroblast growth factor | $10^{-6}$ M | 28 | 0 |

Rates of protein degradation were determined as described in the text and as shown graphically in Figure 1. Degradation was followed for 4-20 hours and compared to values obtained for cells incubated in DMEM alone. The askerisk (*) signifies inhibition values obtained from earlier work in our laboratory (20).

Table 2 shows that ginseng and insulin also stimulate protein synthesis by approximately 30% in human fibroblasts. Similar conclusions have been reported for other growth factors (27), but metabolic poisons almost completely inhibit protein synthesis (25,29). These results taken together strongly suggest that ginseng extracts inhibit proteolysis by physiologically relavant mechanisms rather than by nonspecific toxicity.

The components of ginseng that are responsible for promoting protein synthesis and inhibiting proteolysis are not known. Water soluble ginseng extracts contain polypeptides that are stable to boiling (3), and certain of these polypeptides may act as growth factors on fibroblasts in culture. On the other hand, most researchers believe that the active components of

Table 2: The Effect of Ginseng and Insulin on Protein Synthesis

| Medium | N | Protein Synthesis (DPM/ug protein/hr) | % stimulation | p |
|---|---|---|---|---|
| DMEM | 5 | 12.1 $\pm$ 1.0 | --- | --- |
| DMEM + Ginseng | 5 | 15.7 $\pm$ 1.4 | 30 % | $p < .02$ |
| DMEM + Insulin | 5 | 16.1 $\pm$ 1.1 | 33 % | $p < .02$ |

Rates of protein synthesis were determined by incorporation of [$^3$H]leucine into acid-precipitable material over a period of 1 hr as described in Materials and Methods. Ginseng was at a final concentration of 8.7 mg/ml and insulin was $10^{-6}$ M.

ginseng are saponins rather than peptides (2,9,10,13,18). Identifying the active components and establishing how they regulate protein metabolism should lead to a better understanding of the medicinal actions of ginseng.

**Acknowledgements**

We thank Eric Gulve, Mary Ann McElligott, Joseph Berger, and Letitia Beard for many stimulating discussions. This work was supported by grants from the Squibb Corporation and by the National Institutes of Health (AG02783). J.F.D. holds a Research Career Development Award from the National Institute on Aging.

**References**

1. Li, S.C (1596) Pen-T'sao-Kang-Mu, Book III, Volume 12, Commercial Press, Shanghai.
2. Hu, S.-Y. (1977) Am. J. Chin. Med. 5, 1-23.
3. Bae, H-Y. (1978) Korean Ginseng, Korean Ginseng Research Institute.
4. Harding, A.R. (1972) Ginseng and Other Medicinal Plants, A.R. Harding Publishing Co.
5. Wang, B.H. (1980) Acta Pharm. Sinica 15, 312-320.
6. Wang, C.G. and Lei, H.B. (1957) Chin. J. Int. Med. 11, 861-865.
7. Zo, C., Yeung, C.Z., and Lei, H.B. (1959) Acta Pharm. Sinica 7, 28-212.
8. Liu, K.T., Chi, H.C., and Sung, C.Y. (1959) Acta Pharm. Sinica 7, 213-217.
9. Yamamoto, M., Takeuchi, N., Kumagai, A., and Yamamura, Y. (1977) Arzneimittel-Forsch. 27, 1169-1173.
10. Yamamoto, M., Takeuchi, N., and Yamamura, Y. (1977) Arzneim-Forsch. 27,1404-1405
11. Yokozawa, T., Seno, H., and Oura, H. (1975) Chem. Pharm. Bull., Tokyo 23, 3095-3100.
12. Yuan, G.C. and Chang, R.S. (1969) J. Geront. 24, 82-85.
13. Shia, G.T.W., Ali, S., and Bittles, A.H. (1982) Geront. 28, 121-124.
14. Fulder, S.J. (1977) Exp. Gerontol. 12, 125-131.
15. Shibata, Y., Tatsuno, Y., and Higashi, T. (1978) Chem. Pharm. Bull., Tokyo 26, 3832-3835.
16. Kimura, M., Waki, I., Chujo, T., Kikuchi, T., Hiyama, C., Yamazaki, K., and Tanaka, O. (1981) J. Pharmacobiodyn. 4, 410-417.
17. Nagasawa, T., Oura, H., Hiai, S., and Nishinaga, K. (1977) Chem. Pharm. Bull., Tokyo 25, 1665-1670.
18. Oura, H., Hiai, S., Odaka, Y., and Yokozawa, T. (1975) J. Biochem. 77,1057-1065.
19. Ballard, F.J., Wong, S.S.C., Knowles, S.E., Partridge, N.C., Martin, T.J., Wood, C.M., and Gunn, J.M. J. Cell. Physiol. 105, 335-346.
20. Auteri, J.S., Okada, A., Bochaki, V., and Dice, J.F. (1983) J. Cell. Physiol. 115, 167-174.
21. Dice, J.F. (1982) J. Biol. Chem. 257, 14624-14627.
22. Bradley, M.O., Hayflick, L., and Schimke, R.T. (1976) J. Biol. Chem. 251, 3521-3529.
23. Janeczko, R.A. and Etlinger, J.D. (1984) J. Biol. Chem. 259, 6292-6297.
24. Knowles, S.E. and Ballard, F.J. (1976) Biochem. J. 156, 609-617.
25. Poole, B. and Wibo, M. (1973) J. Biol. Chem. 248, 6221-6226.
26. Hershko, A., Mamont, P., Shields, P., and Tomkins, G. (1971) Nature 232,206-211.
27. Gunn, J.M., Bodner, J.B., Knowles, S.E., and Ballard, F.J. (1983) Biochem. J. 210, 251-258.
28. Neff, N.T., DeMartino, G.N, and Goldberg, A.L. (1979) J. Cell. Physiol. 101, 439-458.
29. Gronostajski, R.M., Goldberg, A.L., and Pardee, A.B. (1984) Mol. Cell. Biol. 4, 442-448.