EFFECTS OF CYTOTOXIC FRACTIONS OF KOREAN GINSENG ROOTS ON MACROMOLECULAR SYNTHESIS IN CANCER CELLS

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Extracts of *Panax ginseng* have been known to stimulate RNA and protein synthesis in rat liver and kidney *in vivo*[1,2,3]. On the other hand, several papers concerning the cytotoxic activities of ginseng extracts have been reported and certain fractions shown to be inhibitory to the macromolecular synthesis in mammalian cells[4,5]. It was also reported that extracts of *Panax ginseng* roots exhibited anticancer activities[6,7].

The present study was undertaken to obtain more information on these cytotoxic fractions from Korean ginseng roots. In this paper, we describe effects of two cytotoxic fractions on macromolecular synthesis in mouse Sarcoma 180 cells *in vitro*. One of these fraction(fraction I) is the non-saponin fraction described by B. H. Han of Natural Products Research Institute, Seoul National University, Korea and the other(fraction II, the petroleum ether fraction) described by W. I. Hwang of College of Medicine, Korea University, Korea and S. M. Cha of Brown University, U.S.A.

Materials and Methods

Cells—Cultured L5178Y cells in the exponential phase of growth in Fischer's medium supplemented with horse serum(FMS) were used to quantify the cytotoxic activity. Mouse Sarcoma 180 ascites cells in FMS were used for the studies on macromolecular synthesis. They were maintained by weekly transfer of about one million cells into the peritoneum of Swiss Webster mice. The cell number was determined in a Coulter counter. Ginseng extracts—the fraction I was obtained from B.H. Han. It was extracted from Korean ginseng roots by alcohol. The fraction II was kindly supplied by W. I. Hwang. It is petroleum ether extract of Korean ginseng roots.

Methods—The cytotoxicity was measured by outgrowth method. The effects of ginseng fractions on the macromolecular synthesis were traced by labelling cells with radioactive precursors. Synthesis of individual RNA species was followed by fractionation of subcellular components and purification and separation of RNA by the sucrose gradient centrifugation. Effects of ginseng fractions on protein synthesis were also studied by examining polyribosomal patterns on sucrose gradient after centrifugation of cytoplasmic extract. Methods of subcellular fractionation and purification and separation of individual RNA species were adapted from the methods developed for Sarcoma 180 cells by Lee *et al.*[8]. Pretreatment by 0.04 μg/ml actinomycin D was used when effects of ginseng fractions on non-ribosomal RNA species were followed.

Results and Discussions

Cytotoxic effects of fraction I and fraction II

The inhibitory effect of fraction I on L5178Y cell growth *in vitro* was evident as shown in Fig. 1. The cytotoxic effect was due to the acute cell death. The loss of cell viability was approximately exponential to the extract concentration. Similar effects were observed with fraction II as reported by Hwang and Cha[5].

Effect of fraction I on DNA, RNA and protein synthesis

Effects of fraction I on the incorporation of radioactive exogeneous precursors into DNA, RNA and protein in Sarcoma 180 cells were studied at different concentrations of fraction I (Fig. 2).

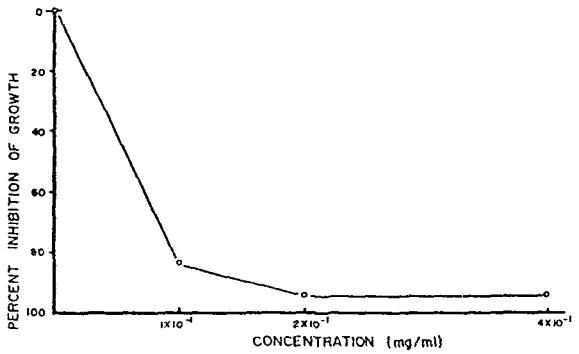

Fig. 1. Dose-dependent growth inhibition of L5178Y cells caused by fraction I from Korean ginseng roots in suspension culture.

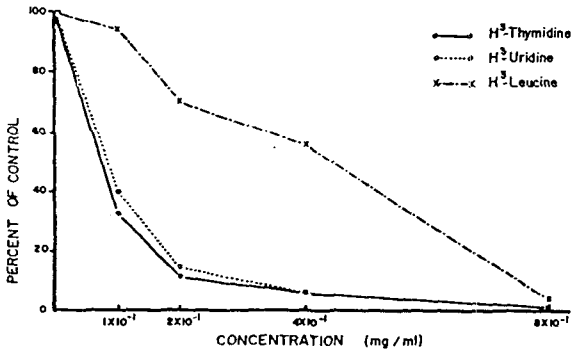

Fig. 2. Effect of fraction I from Korean ginseng roots on DNA, RNA and protein synthesis as a function of concentration in Sarcoma 180 cells *in vitro*.

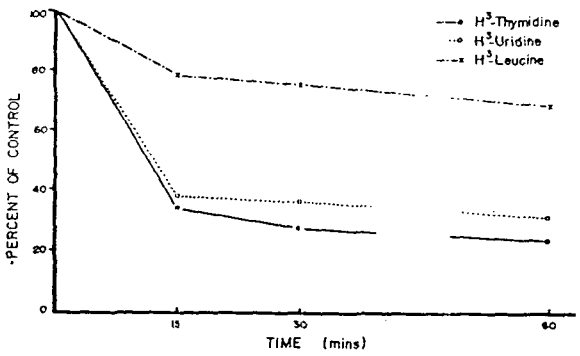

Fig. 3. Effect of fraction I from Korean ginseng roots on DNA, RNA and protein synthesis as a function of time in Sarcoma 180 cells *in vitro*.

The results of a time course experiment are presented in Fig. 3. Synthesis of DNA and RNA was more sensitive to fraction I than protein synthesis. The inhibitory effects were approximately exponential to the extract concentration but affected only a little by the duration of drug treatment. The cytotoxic effect of fraction I seems to be well correlated with the inhibitory effect on DNA and RNA synthesis although cells used in the two experiments were different.

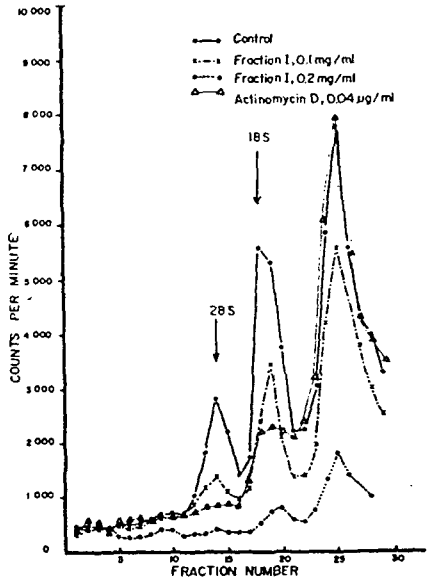

Fig. 4. Effect of fraction I from Korean ginseng roots on cytoplasmic RNA of Sarcoma 180 cells.

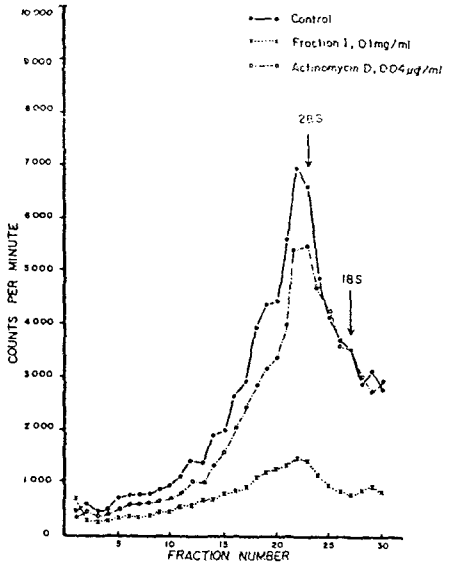

Fig. 5. Effect of fraction I from Korean ginseng roots on nuclear RNA of Sarcoma 180 cells.

The effect of fraction I on the cytoplasmic RNA of Sarcoma 180 cells is shown in Fig. 4. The synthesis of ribosomal RNA, messenger RNA and transfer RNA was almost equally affected by fraction I, although at lower extract concentration the ribosomal RNA synthesis seems to be slightly more susceptible to the drug than the rest of RNA species. The heterogeneous nuclear RNA(non-inhibitory to 0.04 μg/ml actinomycin D) was the most susceptible to fraction I treatment (Fig. 5).

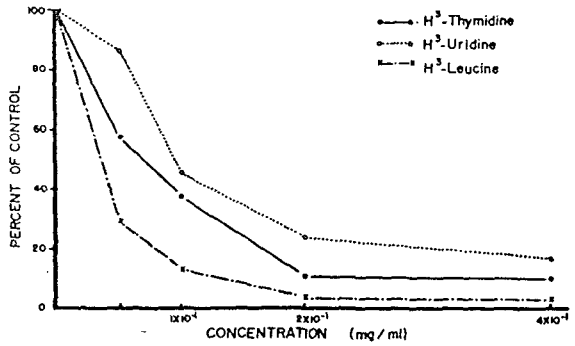

Fig. 6. Effect of fraction II from Korean ginseng roots on DNA, RNA and protein synthesis as a function of concentration in Sarcoma 180 cells *in vitro*.

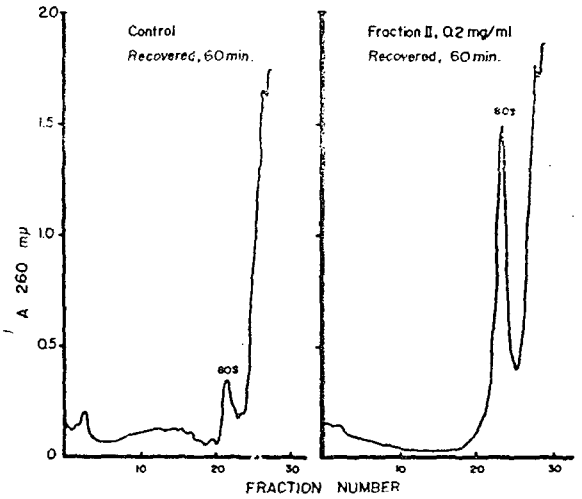

Fig. 7. Effect of fraction II from Korean ginseng roots on polysome pattern of Sarcoma 180 cells.

Effect of fraction II on DNA, RNA and protein synthesis

Effects of fraction II on the incorporation of radioactive exogeneous precursors into DNA, and protein in Sarcoma 180 cells at different concentrations of fraction II is shown in Fig. 6. On the contrary to fraction I, fraction II inhibited protein synthesis more than DNA or RNA synthesis. This clearly indicates that the two fractions contain different active component. The inhibitory effect on protein synthesis was due to the interference in polysome formation (Fig. 7). The initiation step of protein synthesis seems to be blocked by the fraction II treatment rather then elongation step, since

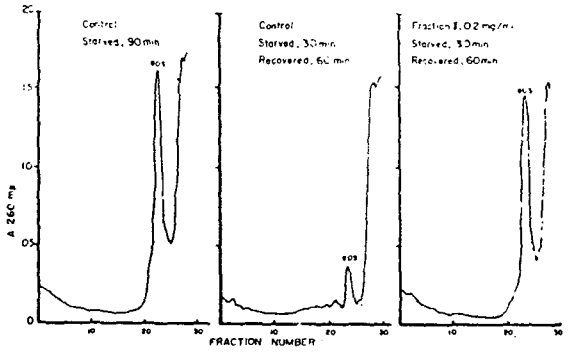

Fig. 8. Effect of fraction II from Korean ginseng roots on polysome formation of Sarcoma 180 cells.

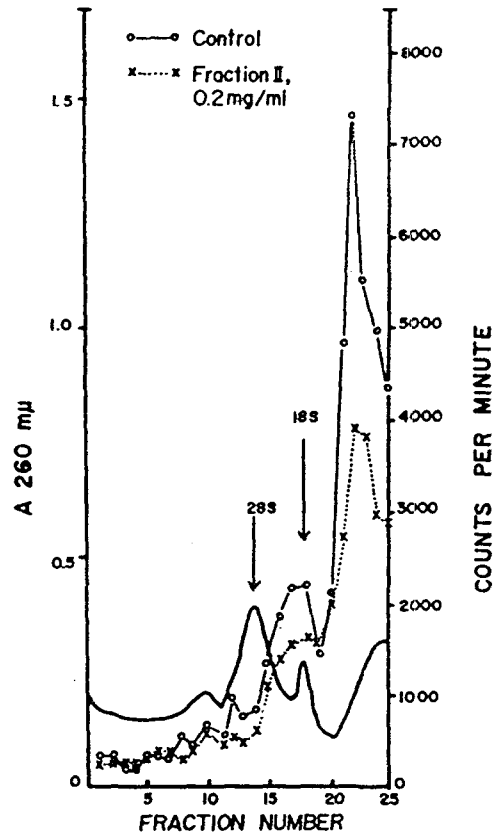

Fig. 9. Effect of fraction II from Korean ginseng roots on the stability of cytoplasmic RNA of Sarcoma 180 cells.

fraction II stimulated the dissociation of polysomes (Fig. 7) and prevented the formation of polysomes from monosomes when the cells were recovered from amino acid starvation (Fig. 8). The possibility of messenger RNA degradation by fraction II treatment was also excluded because the pre-labelled messenger RNA profiles in both treated and untreated cells looked similar(Fig. 9).

Conclusions

1. Fraction I and II contain cytotoxic activities differing their inhibitory effects on macromolecular synthesis in Sarcoma 180 cells. Fraction I inhibits DNA and RNA synthesis more than protein synthesis while fraction II inhibits protein synthesis preferably.
2. The inhibitory effect of fraction I on RNA synthesis in Sarcoma 180 cells *in vitro* is relatively non-specific among RNA species. The heterogeneous nuclear RNA is slightly more susceptible than the rest of RNA species.
3. The inhibitory site of fraction II on protein synthesis is the initiation step.

References

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