Effect of Crystalline Abrin on the Biosynthesis of Protein, RNA, and DNA in Experimental Tumors

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SUMMARY

Crystalline abrin isolated from Abrus precatorius L. has been shown to have a strong inhibitory effect on the protein biosynthesis of Ehrlich ascites tumor and Yoshida ascites hepatoma cells and a moderate inhibitory effect on the DNA biosynthesis of these neoplastic cells. It has no significant effects on the RNA biosynthesis and cellular respiration of these neoplastic cells at a much higher concentration of abrin than that required for curing the tumor. The inhibitory effect of abrin was not significantly changed by addition of glucose. By heating the solution of abrin at 100°C for 30 min, the inhibitory effect of abrin was completely abolished.

INTRODUCTION

Abrin, a highly toxic protein, has been isolated and crystallized in our laboratory (5). In the earlier studies, the crystalline abrin had shown remarkable effects on the biosynthesis of protein and DNA of poisoned rat livers but no effect on the mitochondrial respiration (3, 6). Since abrin inhibits macromolecule biosynthesis, it has been tested for its effect on Ehrlich ascites tumor, and the results showed that the growth of Ehrlich ascites tumor was inhibited completely when a single sublethal dose of abrin was injected i.p. immediately after inoculation of the tumor cells (4). In further studies, it was demonstrated that abrin also suppressed the growth of Ehrlich ascites tumor when abrin was injected i.p. on the 8th day after tumor transplantation, but 2 doses were required (7). By determining the number of tumor cells in the intraperitoneal cavity of each mouse, the results indicated that abrin caused a cytolytic effect on the tumor cells (4).

These findings led us to seek explanations for the cytotoxic effect of abrin on the growth of the tumor cells. In the present work, we have studied the effect of abrin on the biosynthesis of protein, RNA, and DNA, as well as its effect on the oxidation of glucose in the tumor cells.

MATERIALS AND METHODS

Animals and Transplantation of Tumors. Adult male mice of NMRI strain, weighing 20 to 25 g, and male albino rats of the Sprague-Dawley strain, weighing 200 to 250 g, were used for these experiments. Ehrlich ascites tumor was maintained by i.p. transplantation of 0.2 ml of ascites fluid containing 2 × 10^6 to 4 × 10^7 cells into recipient NMRI strain mice every 3 weeks (4, 7). Transplantation of Yoshida ascites hepatoma was carried out by withdrawing ascites fluid from donor rats bearing a weak growth of Yoshida ascites hepatoma cells. A 0.1-ml portion of the ascitic fluid, containing 7.5 × 10^6 to 10^7 cells with 95% viability as determined by the trypan blue dye exclusion method, was injected i.p. into each rat.

Determination of Protein. The amount of protein in the samples was quantitatively determined by the Folin phenol method of Lowry et al. (8).

Preparation of Abrin. Crystalline abrin was isolated and purified as described previously (5). It was dissolved in cold Krebs-Ringer phosphate buffer to various concentrations for the following experiments.

Isotopic Compounds and Incubation of Ascites Tumor Cells. Thymidine-methyl^3H with a specific activity of 6.7 Ci/m mole, uridine-2-^14C with a specific activity of 55.3 mCi/m mole, and L-leucine-4,5-^3H with a specific activity of 5 Ci/m mole were purchased from New England Nuclear Corp., Boston, Mass.

A 5-ml portion of ascitic tumor fluid containing about 10^8 cells/ml was centrifuged in 15-ml centrifuge tubes in an International refrigerated centrifuge at 500 x g for 2 min at 2°C, and the packed cells were washed 3 times with ice-cold Krebs-Ringer phosphate buffer. Five ml of the diluted cell suspension (10^7 cells/ml) was placed in a 25-ml Erlenmeyer flask containing 0.2 ml of indicated amount of ice-cold abrin solution. The flasks were placed in a Eberbach water bath shaker (95 to 100 oscillations/min) at 37°C for 30 min. Then, the supernatant was removed by centrifugation, and the packed cells were washed 3 times with Krebs-Ringer phosphate buffer. Finally, abrin-treated cells were incubated at 37°C for 5 min in an International refrigerated centrifuge at 0°C for 2 min at 2°C, and the packed cells were washed 3 times with Krebs-Ringer phosphate buffer. The filter paper discs for TCA precipitation were allowed to equilibrate at 0°C. After equilibrium, 0.2 ml each of L-leucine-4,5-^3H (10 μCi/ml), uridine-2-^14C (1 μCi/ml), or thymidine-methyl^3H (10 μCi/ml) in Krebs-Ringer phosphate buffer were added to each reaction flask to make a final volume of 2 ml (2.5 × 10^6 cells/ml), and the samples were further incubated for 30 min. The reaction was terminated by adding 0.2 ml of 10% TCA followed by cooling to −25°C.

Determination of Radioactivity. The radioactivity of the samples was determined by the filter paper disc method (9). Aliquots of samples were pipetted onto filter paper discs.
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The results of the effects of abrin on the biosynthesis of protein, RNA, and DNA of Yoshida ascites hepatoma are similar to that of Ehrlich ascites tumor.

In the previous experiments, abrin was preincubated with tumor cells for 30 min. To study whether it was essential for abrin to be present continually in order to exhibit its inhibitory effect, the tumor cells were preincubated with abrin for various periods of time and then washed and transferred into abrin-free Krebs-Ringer phosphate buffer containing the isotopic compounds. The results are presented in Chart 2. Control Ehrlich ascites cells incubated in Krebs-Ringer phosphate buffer maintained their ability to synthesize protein, RNA, and DNA. However, incubation of cells in the same buffer system in the presence of 2 μg/ml of abrin resulted in a rapid loss of ability to synthesize tissue, and, after 1 hr, less than 5% of protein biosynthesis of that of control remained. For DNA biosynthesis, the inhibition caused by abrin reached its maximum at 1 hr; thereafter, there was no further marked increase in inhibition. Abrin did not show any significant inhibitory effect on RNA biosynthesis for 3-hr incubation (Chart 2).

We further studied whether abrin interferes with protein biosynthesis by inhibiting glucose metabolism. This was carried out by adding increasing amounts of glucose (2 to 100 μmoles/ml) to the incubation mixture. The results show that added glucose did not reverse the inhibitory effect of abrin. The inhibitory effects of abrin on the biosynthesis of protein and DNA were completely prevented by heating abrin solution.

RESULTS

Since the incorporation of radioactive leucine, uridine, and thymidine into the insoluble fraction of tumor cells in the absence of abrin was linear for more than 1 hr, an incubation time of 30 min was adopted in these experiments. The effects of abrin on the biosynthesis of macromolecules were studied, and the results are summarized in Chart 1. Abrin at a very dilute concentration, 1 to 2 μg/ml, almost completely inhibited the biosynthesis of protein of Ehrlich ascites tumor cells and inhibited about 40% of DNA synthesis, but it had no effect on the RNA biosynthesis at this low concentration. When the concentration of abrin was increased to 10 μg/ml, abrin still did not show any significant inhibitory effect on uridine incorporation into Ehrlich ascites tumor cells.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{chart1.png}
\caption{Chart 1. Inhibitory effect of abrin in vitro on the incorporation of L-leucine-3H, uridine-14C, and thymidine-3H into the Ehrlich ascites tumor cells. Incubation of normal or abrin-treated tumor cells with radioactive compounds and preparation of samples for radioactive assay were as given in “Materials and Methods.” The incorporation of radioactive leucine, uridine, and thymidine into the insoluble fraction of tumor cells in the absence of abrin were: 5.6 × 10^3 cpm/mg protein for protein, 4.5 × 10^4 cpm/mg protein for DNA, and 1.4 × 10^4 cpm/mg protein for RNA. α, uridine-14C; α, thymidine-3H; X, leucine-3H.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{chart2.png}
\caption{Chart 2. Inhibitory effect of preincubation of abrin in vitro on the incorporation of L-leucine-3H, thymidine-3H, or uridine-14C into the TCA-insoluble fraction of Ehrlich ascites tumor cells. For each time point, 5 × 10^6 tumor cells in 5 ml of Krebs-Ringer phosphate buffer were preincubated with abrin (2 μg/ml) at 37°. At the predetermined times, the reaction mixture was cooled and centrifuged at 500 × g at 2° for 2 min in an International refrigerated centrifuge. The supernatant was removed, and the cells were washed 3 times, each time with 10 ml of ice-cold Krebs-Ringer phosphate buffer. Finally, the washed cells were resuspended in 5 ml Krebs-Ringer phosphate buffer, pH 7.4. After equilibration at 37° for 5 min, 2 μCi of L-leucine-3H, 2 μCi of thymidine-3H, or 0.2 μCi of uridine-14C were added to 2 ml of cell suspension containing 5 × 10^6 cells, and the cell preparation was incubated at 37° for 30 min. The controls were done as above, except that abrin was not added. For preparation of samples for radioactivity assay, see “Materials and Methods.” The degrees of incorporation of radioactive leucine, uridine, and thymine into insoluble fraction of control tumor cells are the same as those of Chart 1. α, uridine-14C; α, thymidine-3H; X, leucine-3H.}
\end{figure}
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at 100° for 30 min. Therefore, the inhibitory effect of abrin is protein in nature.

The possibility that abrin inhibits the growth of tumor cells by preventing cellular respiration was studied with a Warburg apparatus. There is a linear relationship between cell number and oxygen uptake. The oxygen consumption increases with increase of cell number. Abrin did not inhibit the oxygen uptake of tumor cells when it was added at 0 time. With from 0.5 to 3 hr preincubation of abrin with tumor cells, the respiration of treated tumor cells was the same as that of intact tumor cells. Therefore, the metabolic changes provoked by abrin probably do not depend on effects of abrin on the respiration of tumor cells.

**DISCUSSION**

The fact that the incorporation of leucine was inhibited much more strongly than that of thymidine and that there was no inhibition of incorporation of uridine suggests that the effect of antitumor activity of abrin is due to its specific reaction in the biosynthesis of protein. The fact that addition of increased amounts of glucose did not prevent the inhibition caused by abrin suggests that the inhibitory effect of abrin on cell metabolism is not due to the impairment of D-glucose phosphorylation. It also has been shown (3, 6) that abrin inhibits the protein and DNA biosynthesis of poisoned rat liver, but not mitochondrial respiration. In vitro, we were also able to demonstrate that abrin did not affect the respiration of tumor cells, while under the same conditions the protein biosynthesis of tumor cells was completely prevented.

The cytotoxic effect of the extract of the seeds of *A. precatorius* L. has been demonstrated (10); it induced vacuolation and disruption of cytoplasm accompanied by karyolysis and chromosomal abnormalities in Yoshida ascites hepatoma cells. In these experiments, the antitumor effects of abrin are demonstrated at the molecular level, which suggests that abrin exerts its cytotoxic effect on the tumor cells by inhibiting the protein and DNA biosynthesis of tumor cells.

In the reports of earlier workers (1, 2, 10), there was no conclusive result about the antitumor activity of the active principle from the extracts of *A. precatorius* L. The antitumor activity of crystalline abrin was shown unequivocally in our early reports (4, 7), and its possible mechanism of antitumor activity is demonstrated in the present data.

**REFERENCES**

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