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THE EFFECT OF THE ETHYL HYDRAZIDE OF PODOPHYLLIC ACID (SP I SANDOZ) ON PROTEOSYNTHESIS OF NORMAL AND TUMOUR TISSUES *IN VIVO*

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Abstract—The effect of ethyl hydrazide of podophyllic acid (SP I Sandoz) on protein synthesis *in vivo* was investigated by measuring the rate of incorporation of ^{14}C-labelled amino acids into proteins of the serum, liver, and tumour tissue of rats. Following a single dose of 100 mg/kg of SP I administered intravenously, at the end of 1 hr after application, a 50 per cent inhibition of protein synthesis was found in transplanted RB I tumour. Serum protein synthesis and protein synthesis in the liver were not inhibited at this SP I dose. Liver and serum protein synthesis was not lowered sooner than after a single dose as high as 150 and 200 mg/kg respectively. Following an i.v. dose of 200 mg/kg body weight there was a 75 per cent protein synthesis inhibition as compared with control animals.

In rats which received repeated single daily doses of 50 mg/kg for a period of 5 months, an inhibition of serum protein synthesis of about 30 per cent was seen; also an inhibitory action on proteosynthesis in the liver was observed in these animals.

ETHYL hydrazide of podophyllic acid (SP I Sandoz) has been recently used extensively as a cytostatic for the treatment of malignant tumours.[1–3] From behaviour, this drug ranks among antimitotic agents like the derivatives of colchicine and vinblastine. It has been demonstrated experimentally that SP I arrests cell mitosis in metaphase.[4] The biochemical mechanisms of the action of SP I on malignant cell is still unknown.

In the present paper we present the results obtained by investigating the effect of SP I on protein synthesis in liver and tumour tissues of rats *in vivo*.

MATERIALS AND METHODS

Animals

The experiments were carried out on rats of the Wistar and Sprague–Dawley strains.

Tumours

The following tumour was used in the experiments: transplanted solid rat sarcoma RB I.[5]

Chemical agents used

Ethyl hydrazide of podophyllic acid (SP I) was kindly supplied by courtesy of Sandoz, A.G., Basel, Switzerland. Algal protein hydrolysate—^{14}C (U) (32·3 mc/g)—a preparation of the Institute for Radioisotope Production and Utilization, Prague,

was used for amino acid incorporation studies. Proteins were determined by the method of Lowry and co-workers.[6]

Incorporation of ^{14}C-*amino acids into proteins of the liver and tumour* in vivo

Following the injection of the cytostatic, the experimental animals were administered intraperitoneally algal protein hydrolysate-^{14}C (U) in the dose of 20 μc/rat. At the end of 1 hr the animals were sacrificed and the tissue was quickly removed. A 5 per cent suspension was prepared from the tissue by homogenization in 0·01 M NH_4HCO_3 buffer, pH 8·6. The homogenate was centrifuged at 3000 *g* for 5 min. The supernatant was precipitated by adding an equal vol. of 10% trichloracetic acid, the sediment was washed three times with 5% trichloracetic acid, three times with 95% ethanol, and finally dissolved in concentrated formic acid. Radioactivity was measured with a thin-end window Geiger–Müller tube.[7]

The same results were obtained by removing free amino acids of the supernatant on a Sephadex G 25 column.

RESULTS

Healthy rats, weighing 200 g on an average, received repeated single i.p. doses o 50 mg/kg of SP I for a period of 5 months. After this treatment, incorporation of ^{14}C-amino acids was determined in these animals as the measure of proteosynthesis *in vivo*. The results obtained are presented in Table 1. Besides histopathological changes

TABLE 1. EFFECT OF CHRONIC ADMINISTRATION OF SP I ON THE SERUM AND LIVER PROTEIN SYNTHESIS OF RATS

Material		No. of animals	Counts/min per mg of protein	Inhibition %
Serum	control	6	46·2 ± 1·43	0
	treated	6	31·2 ± 1·38	32·4
Liver	control	6	49·7 ± 1·82	0
	treated	6	39·9 ± 1·1	19·8

which will be the object of another communication, chronic administration of SP I causes a moderate inhibition of synthesis of serum proteins, and exerts an inhibitory effect also on the biosynthesis of liver proteins.

The results obtained in investigating the effect of i.v. injections of SP I on the incorporation of ^{14}C-amino acids into proteins of the serum, liver and tumour tissue of rats are shown in Fig. 1. A single dose of 100 mg of SP I/kg of body weight of the animal brings about, 1 hr after application, inhibition of protein synthesis in the tumour while liver and serum protein synthesis remains uninfluenced. A higher dose (150–200 mg/kg) inhibits synthesis of the proteins of the liver and serum, but with high doses protein synthesis inhibition in the tumour is about twice as high as the inhibition of protein synthesis in the liver.

On i.p. injection of SP I in doses of 200 mg/kg to tumour-bearing rats of various ages (Table 2) SP I was found to inhibit protein synthesis of the liver and serum to

TABLE 2. EFFECTS OF SINGLE DOSE OF 200 mg/kg i.p. ON THE SYNTHESIS OF SERUM, LIVER AND TUMOR PROTEINS OF RATS *in vivo*

Material		Body wt. = 35 g			Body wt. = 50 g		
		No. of animals	Counts/min per mg of protein	Inhibition (%)	No. of animals	Counts/min per mg of protein	Inhibition (%)
Serum	Control	2	166 ± 8·0	0	4	119 ± 5·65	0
	Treated	2	103 ± 9·5	37·9	4	104·5 ± 9·6	24·5
Liver	Control	2	231 + 13·7	0	4	122·5 ± 2·69	0
	Treated	2	148 ± 8·8	36·0	4	105 ± 6·45	14·4
Tumour	Control	2	118 ± 8·4	0	4	77·6 ± 2·96	0
	Treated	2	30·6 ± 3·4	74·1	4	33·9 ± 1·01	63·5

about an equal degree. Protein synthesis in the tumour is, however, inhibited twice as intensely as compared with the liver and the serum; this is in keeping with results of experiments with i.v. SP I injection. In younger rats the inhibitory effect of SP I is more pronounced.

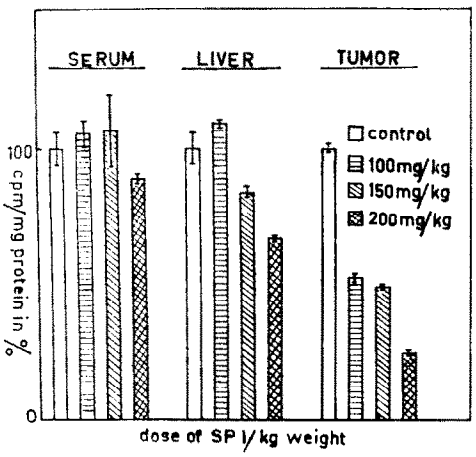

FIG. 1. The effect of varying single i.v. doses on the synthesis of serum, liver, and tumour proteins in rats *in vivo*.
Each value represents a mean of parallel determinations in three experimental and control animals. Mean value ± the average error of arithmetic mean is shown.

DISCUSSION

Judging by the results obtained, ethyl hydrazide of podophyllic acid (SP I) produces at certain doses a specific inhibition of protein synthesis in the tumour as compared with protein synthesis of serum and liver proteins. This specific action of SP I is probably connected with its antimitotic action, and may render it useful in topical perfusion treatment of tumours.[8]

The biochemical mechanism of the observed protein synthesis inhibition *in vivo* under the influence of SP I is not yet known. In preliminary experiments (still not published) when the effect of SP I on the protein synthesis process was investigated in a cell-free system *in vitro*, it was found that the first step of protein synthesis, i.e. the attachment of amino acid on soluble ribonucleic acid remains practically uninfluenced. We observed on a rat model that SP I inhibits the second step of protein synthesis—the transfer of the aminoacyls-RNA complex to ribosomes.

From prophase until the end of telophase, there occurs no deoxyribonucleic acid synthesis. Protein synthesis is minimal, and RNA is synthesized at the beginning and at the end of telophase.[9] We presume that the block of cell mitosis in the metaphase by the action of SP I may be the result of a disorder in ribonucleic acid synthesis. This may find a secondary reflection in the inhibition of protein synthesis.

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