THE EFFECT OF SARCOLYSIN AND DOPAN ON THE BIOSYNTHESIS OF NUCLEIC ACID PYRIMIDINES IN TRANSPLANTED TUMORS AND IN THE TISSUES OF THE RECIPIENT

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It has been shown [1, 3, 5] that the nucleic acid metabolism in the tissues of rats and mice undergoes certain essential changes during the regression of transplanted tumors under the influence of chemotherapeutic drugs.

In order to study the mechanism of these changes, with the assistance of labeled ureidosuccinic acid we investigated changes in the biosynthesis of the nucleic acid pyrimidines in transplanted tumors and in the tissues of recipient rats in response to the action of antitumor drugs, namely sarcolysin and dopan.

Sarcolysin [di-(2-chloroethyl) aminophenylalanine], when administered to rats with sarcoma 45 in certain doses, brings about complete absorption of this sarcoma [4]. A dose of 30 mg/kg body weight is toxic, and leads to death of rats on the 4th-5th day after administration. When performing experiments with sarcolysin in vivo we selected this dosage, in order to secure the maximum possible action of the drug on the processes under investigation. Dopan [4-methyl-5-di(2-chloroethyl) aminouracil] causes lasting absorption of sarcoma 45 in 90% of rats so treated [2].

In experiments in vitro we gave sarcolysin and dopan in doses considerably larger than therapeutic, in order to exceed the threshold of ineffective combination of the drugs with the tissue.

METHOD

Ureidosuccinic acid (US), with the ureide carbon labeled with C^{14}, was synthesized by the method of Nyc and Mitchell [6]. The relative activity of the US thus obtained was 1.9 mC.

For the experiments we selected male rats weighing up to 110 g. Sarcolysin and dopan were given on the 12th day after transplantation of a sarcoma 45. Sarcolysin was injected intraperitoneally as a single dose of 30 mg/kg. Dopan was given by mouth in the form of a suspension in 1% starch solution, in three doses of 0.75 mg/kg body weight at intervals of 72 hr. The animals were sacrificed 72 hr after the third dose. Radioactive US was injected subcutaneously or intraperitoneally in a neutral aqueous solution.

After a single injection of US into the rats the radioactivity per gram body weight was 7000-10,000 cpm; after repeated injection at intervals of three and 18 hours between injections, in order to produce gradual accumulation of radioactivity in the animals, the total dose of radioactivity injected was 25,000-28,000 cpm/g body weight. The radioactivity of the specimens was measured by means of the MST-17 end-type counter and a B-2 apparatus. Animals were sacrificed 2 hr after injection of US.

The tissues for investigation were treated by the method of Schmidt and Thanhauser [7]. A weighed sample of tissue was minced and ground up with five times its own weight of 5% trichloroacetic acid (TCA) or with 0.2 N $HClO_4$. The radioactivity of the supernatant fluid obtained after centrifugation (subsequently called the acid-soluble fraction) was determined after neutralization and suitable treatment. The lipids were extracted from the precipitate; the residue, containing nucleoproteins, was dried with alcohol and ether, and analyzed, and hot extraction of the nucleic acids in a separate portion of the residue was carried out with 5% TCA or 0.2% $HClO_4$.

The results obtained are shown either as a ratio between the radioactivity detected in impulses (x 100) per 10 mg of the specimen and the radioactivity injected per gram body weight, or as impulses per 10 mg of specimen. The action of the antitumor preparations was investigated in vitro and in vivo.

RESULTS

Effect of sarcolysin in vitro. These experiments were carried out on sections of liver and sarcoma 45, obtained from rats on the 12th day after transplantation. In the experiment we used 0.5 g of tissue sections, sarcolysin solution in a dose of 100 mg/kg and US solution with an activity of 100,000 impulses to a test sample of 5 ml. The samples were incubated in Krebs-Ringer solution for 60-90 min in a water bath at a constant temperature of 37°, with agitation.

TABLE 1. The Effect of Sarcolysin on the Incorporation of US in the Nucleoproteins of Sections of Liver and Sarcoma 45 in 45 Rats (in impulses per 10 mg of the specimen)

Experiment No.	Liver		Sarcoma 45	
	control	experiment	control	experiment
1	68	74	113	103
2	79	75	158	138
3	57	62	89	67
4	52	48	83	75
5	—	—	34	30

It will be seen from the figures in Table 1 that sarcolysin had no effect on the incorporation of US by the liver sections of the recipient rats. The effect of sarcolysin on the incorporation of US by the nucleoproteins of the sections of sarcoma 45 was slight, although in the five experiments illustrated it was shown in the same direction, namely as a lowering of the intensity of incorporation on the average by 10 %.

Effect of sarcolysin in vivo. Sarcoma 45 was transplanted into rats, and the animals then received sarcolysin by intraperitoneal injection in a dose of 30 mg/kg. The animals were sacrificed three and 24 hours after the injection of sarcolysin and two hours after injection of US. Control rats, in which tumors had been transplanted, were sacrificed at the same times (Table 2).

TABLE 2. The Effect of Sarcolysin on the Incorporation of US in the Nucleoproteins of the Tissues of Rats with Sarcoma 45 (24 hours after injection)

Tissues	Nucleoproteins		Acid-soluble compounds		Nucleic acids	
	control	experiment	control	experiment	control	experiment
Liver	239 0,94	361 1,46	292 1,14	433 1,75	180 0,71	240 1,00
Mucous membrane of the small intestine	57 0,22	40 0,16	48 0,18	13 0,05	16 0,06	16 0,06
Sarcoma 45	17 0,07	26 0,10	16 0,06	16 0,06	7 0,02	8 0,03

Note: Upper row of figures — impulses per 10 mg of specimen; lower — relative activity.

In Table 2 are shown the mean results of three experiments carried out on rats in which the same tumor was transplanted. It follows from these results that an obvious increase in the intensity of biosynthesis of pyrimidines from US, of roughly 25-34%, took place in the liver under the influence of sarcolysin in all the fractions investigated. In the mucous membrane of the small intestine the absolute values of the radioactivity were small, although it could be noticed that the incorporation of US into the fractions of nucleoproteins and acid-soluble compounds was diminished under the influence of sarcolysin. Practically no changes were found in sarcoma 45.

At sacrifice of the rats three hours after injection of sarcolysin, the changes in these particular tissues were in the same direction as at the investigation after 24 hours.

In rats with ascitic hepatoma (Zazhdel' strain), after injection of sarcolysin in a dose of 30 mg/kg into the ascitic fluid, 22 hours after injection an increase in the incorporation of US into the nucleoproteins of the liver by an average of 40 was also observed (Table 3).

TABLE 3. The Effect of Sarcolysin on the Incorporation of US in the Nucleoproteins of the Liver and Ascitic Fluid of Rats (22 hours after injection)

Tissues	Nucleoproteins	
	control	experiment
Liver	304	420
Ascitic fluid .	43	35

Note: In impulses per 10 mg of specimen. Mean of four experiments

TABLE 4. The Effect of Dopan on the Incorporation of US in the Nucleoproteins of Sections of Liver and Sarcoma 45

Experiment No.	Liver		Sarcoma 45	
	control	experiment	control	experiment
1	18	18	58	54
2	21	18	60	50
3	20	24	50	60
4	18	24	—	—

Note: In impulses per 10 mg of specimen.

Effect of dopan. The elucidation of the effect of dopan on the biosynthesis of pyrimidines from US was of especial interest, for dopan is a chloroethylamino-derivative of uracil, and it might have been expected that during interaction with US, the precursor of the pyrimidines, competitive relationships might have arisen during the synthesis of nucleic acids. Experiments were carried out in vitro on sections of liver and sarcoma 45. Dopan was added in a dose of 100 mg/kg body weight (0.05 mg dopan corresponding to 500 mg of sections) in the form of a suspension in Krebs-Ringer solution. US

TABLE 5. The Effect of Dopan on the Incorporation of US in the Nucleoproteins of the Tissues of Rats with Sarcoma 45

Tissue	Nucleoproteins		Acid-soluble compounds	
	control	experiment	control	experiment
Liver	314 1,44	436 1,89	347 1,58	525 2,26
Mucous membrane of the small intestine	54 0,24	61 0,26	— —	— —
Sarcoma 45	31 0,14	55 0,23	46 0,21	73 0,31

Note: Upper row of figures -- impulses per 10 mg of specimen; lower -- relative activity.

with a total radioactivity of 65,000 cpm was added to the 5 ml samples.

Incubation was for one hour in a water bath at a constant temperature of 37° with agitation (Table 4).

It may be seen from the figures in Table 4 that in our experimental conditions in no case had dopan any effect on the incorporation of US in the nucleoproteins of the sections of liver and sarcoma 45.

In the experiments in vivo, dopan was given on three occasions, as described in the section on method. The results obtained are shown in Table 5.

The dopan experiments on intact animals showed that the same intensification of biosynthesis of the pyrimidines, of the order of 35-40%, takes place in the nucleoproteins of the liver under the influence of dopan as was observed under the influence of sarcolysin. It was also found that the incorporation of US in the nucleoproteins of sarcoma 45 is increased as a result of the action of dopan.

The information now available on the mechanism of action of sarcolysin and dopan (only part of which has been published [3]) supports the point of view that injury of the nucleic acid metabolism is one of the main, but probably not the only, links in the mechanism of action of antitumor drugs with chloroethylamino-groups. Whereas the action of antitumor drugs of the antimetabolite group is characterized by their action on a particular and narrowly specific link in metabolism, that of the drugs under investigation, with an alkyl group, is evidently characterized by a wide field of interaction with many links in metabolism.

According to our findings, sarcolysin and, what is especially interesting, dopan do not lead to any specific injury to the biosynthesis of pyrimidine-nucleic acids from US as a precursor.

SUMMARY

The author studies the effect of sarcolysin and dopan on the synthesis of nucleic acid pyrimidines from the ureidosuccinic acid (with C^{14}-labeled ureide carbon) in the tissues of rats with sarcoma 45 and ascitic hepatoma. The incorporation of ureidosuccinic acid in the nucleoproteins of the liver is increased by sarcolysin. The utilization of ureidosuccinic acid by sarcoma 45 and by the cells of the ascitic tumor during the action of sarcolysin and dopan is changed only slightly.

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