THE ACTION OF SARCOLYSIN ON REGENERATION IN AMPHIBIA AND ON TUMORS IN MICE UNDER SLIGHT HYPOTHERMIA

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Investigation of the action of novoembichin [2-chloropropyl-di(2-chloroethyl) amine] and TET (2,4,6-triethylenimino-1,3,5-triazine) on regeneration of the tail of tadpoles after partial amputation showed [4] that these compounds, when injected intraperitoneally, cause considerable inhibion of the regeneration process; their inhibitory effect is, however, considerably weakened if the animals are kept in colder water (14-16°) than that required for optimal conditions of regeneration of the tail (18-22°).

Sarcolysin [p-di(2-chloroethyl) aminophenylalanine], like novoembichin, is a derivative of chloroethylamine, but differs from the latter by having an inhibitory action many times more powerful on the growth of tumors in animals [2]. The inhibitory action of sarcolysin on the reparative regeneration of the liver of the rat is, however, expressed far more weakly than that of other chloroethylamine derivatives [15].

In the present research we studied the distinctive features of the biological action of sarcolysin on the processes of regeneration and growth.

METHOD

Part (1/5) of the tail was amputated in stage II tadpoles of Rana temporaria and Rana ridibunda and, at the same time, the drug was injected intraperitoneally by a method previously described [4], in one single large dose, amounting to not less than 2/5 the lethal dose per kg body weight. In a control group the tail was also amputated, but no drug was injected.

Observations on regeneration lasted 8-10 days. Every 2-3 days the length of the regenerate was measured under the loupe with an ocular micrometer, and the mean value for each group of 25 animals was calculated. These mean values were used to construct a graph of growth and, at the end of the experiment, the percentage inhibition according to the mean length of the regenerate was calculated, i. e. the difference between the mean length of the regenerates in the control and experimental animals, expressed as a percentage of the mean length of the regenerate in the control tadpoles. As an additional control, and in order to compare the action of sarcolysin with that of novoembichin and TET, which had previously been studied on this same experimental object, experiments with these two drugs were conducted at the same time. Altogether 14 experiments were carried out.

RESULTS

In contrast to novoembichin and TET, sarcolysin acts very weakly on regeneration of the tail in ordinary conditions, optimal for the regeneration of this organ. Sarcolysin caused from 11 to 20% inhibition of growth of the regenerate, whereas novoembichin and TET, in parallel experiments with a common control, caused 50-60% inhibition (Fig. 1, 1).

When, however, the midday temperature was lowered by 3-6°, i.e. in cold weather, or when the animals were kept in a refrigerator (at +10°), the inhibitory effect of all three drugs showed obvious changes. Novoembichin and TET almost completely lost their growth-inhibiting properties, and caused about 10% inhibition, whereas sarcolysin, on the other hand, increased its inhibition of growth to from 50 to 80% (Figs. 1, 2 and 3).

It may be postulated from these results that, in animals without a developed thermoregulatory system, the slowing of the metabolic processes by a lowering of the environmental temperature weakens the inhibitory effect of novoembichin and TET on the processes of proliferation (as has been pointed out earlier [4]) and, conversely, increases the inhibitory action of sarcolysin.

An attempt was then made to verify if the relationships concerning the action of these drugs, as shown by a study of regeneration in amphibia, could be applied to the action of the same drugs on the growth of tumors in

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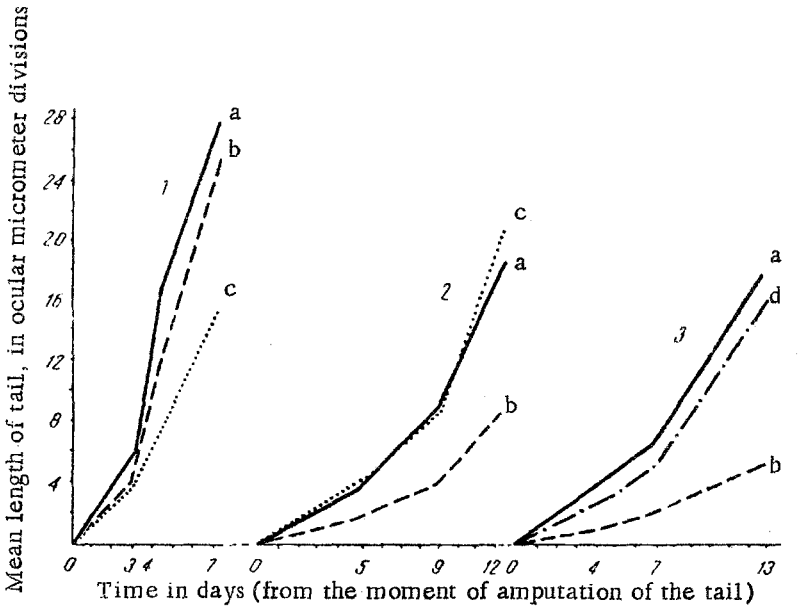

Fig. 1. Dynamics of growth of the regenerating tail of the tadpole of Rana temporaria. a – Control; b – after injection of sarcolysin; c – after injection of novoembichin; d – after injection of TET. 1 – at a midday water temperature of 21°. Doses: sarcolysin 150 mg/kg, novoembichin 6 mg/kg; 2 – at a temperature of 14°. Doses: sarcolysin 50 mg/kg, novoembichin 8 mg/kg; 3 – at a temperature of 12°. Doses: sarcolysin 80 mg/kg, TET 10 mg/kg. I) Mean length of tail, in ocular micrometer divisions II) Time in days (from the moment of amputation of the tail).

mammals. For this purpose an attempt was first made to obtain a strain of Ehrlich's tumor, the cells of which could be adapted more or less permanently to the slower metabolism of a subnormal temperature. Ascitic cells from an Ehrlich's tumor, mixed with citrate, were accordingly kept at 4° for 30 days, after which the mass of cells which settled to the bottom was washed and diluted with physiological saline and then inoculated into mice. The inoculation was carried out intramuscularly into the thigh on one side, and into the thigh of the opposite limb of the same mouse was injected normal, fresh Ehrlich's ascitic cells. It was found that cells which had been subjected to prolonged cooling, like normal cells, caused the development of tumors in 100% of cases, but the tumors were more compact and slower growing than normal. Treatment began on the fifth day after inoculation of the tumor and continued for ten days. Every three days the size of the tumors was measured and the mean tumor diameter for the whole series calculated. From the mean values obtained a graph of tumor growth in the experimental and control animals was constructed (Fig. 2). The degree of inhibition of growth of the tumors was determined as the difference between the mean weight of the tumors in the control and experimental animals, expressed as a percentage of the mean weight of the control tumors. The controls were untreated animals which, like the experimental, received inoculations of normal and cooled strains.

The experiments in which both types of tumors were treated with sarcolysin and TET gave results which were identical in principle with those obtained for these drugs on the regenerating tail in tadpoles. TET, which was injected in a sublethal dose of 3 mg/kg (since in a therapeutic dose it does not generally inhibit growth of an Ehrlich's tumor inoculated intramuscularly), caused

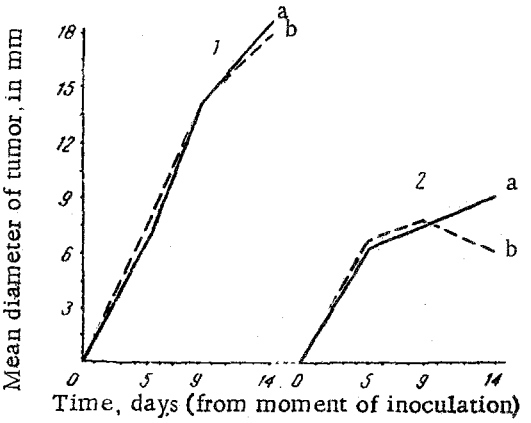

Fig. 2. Dynamics of growth of an Ehrlich's tumor, inoculated intramuscularly. a – Control; b – after injection of sarcolysin (3 mg/kg every 48 hr). 1 – normal strain; 2 – strain treated by cooling. I) Mean diameter of tumor, in mm. II) Time, days (from moment of inoculation).

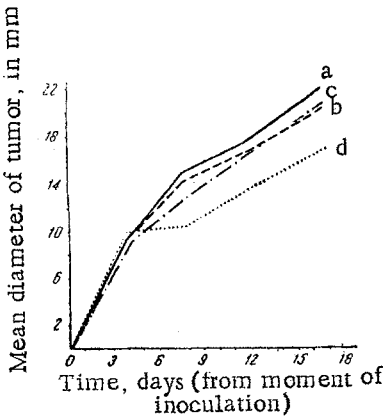

Fig. 3. Dynamics of growth of an Ehrlich's tumor, inoculated intramuscularly. a – Control; b – after injection of sarcolysin (4 mg/kg every 72 hr); c – after injection of chlorpromazine (20 mg/kg daily); d – after injection of chlorpromazine and sarcolysin in the same doses. 1) Mean diameter of tumor, in mm. 2) Time, days (from moment of inoculation).

inhibition of the growth of tumors of the normal strain by 59% and of the strain adapted to cold by 30%. In a therapeutic dose (3 mg/kg every 48 hr), sarcolysin caused the usual slight inhibition for a tumor of the normal strain when inoculated intramuscularly (15%), and on the other hand, a more considerable inhibition (53%) of the adapted strain (see Fig. 2).

When the metabolic processes of the tumor were retarded by adaptation to cold, sarcolysin thus also acquired the power to inhibit the growth of this tumor to a considerable degree, but TET under the same conditions lost this power to a large extent.

In order to potentiate the action of sarcolysin on the tumors we also made use of pharmacological hypothermia of the animal as a whole by means of the injection of chlorpromazine [N(3-dimethylaminopropyl)-2-chlorophenothiazine], which in large doses causes inhibition of oxidative processes in the tissues and a fall in the body temperature of animals for a few hours [8, 10, 11, 13]. On this account, chlorpromazine probably possesses a weak antitumor action [7].

The ordinary course of injections of sarcolysin, after the preliminary injection of large doses of chlorpromazine (20 mg/kg daily, subcutaneously), resulted in 45% inhibition of growth of the tumors in mice with an Ehrlich's tumor inoculated intramuscularly, i.e. more than twice the effect produced by sarcolysin and chlorpromazine when given separately (Fig. 3).

From a comparison of our results with those in the literature on the action of the chloroethylamines and ionizing radiation on the cell, it may be surmised that the activity of the antitumor drugs investigated is dependent on the state of the oxidative processes in the tissues. A subnormal environmental temperature, in the case of cold-blooded animals [9, 12], and chlorpromazine, in the case of mammals [8, 10, 11, 13], are known to lower the intensity of the oxidative processes in the tissues. In accordance with these findings it is possible that the inhibitory action of sarcolysin in our experiments (during hypothermia) is strengthened as a result of the slight anoxia of the tissues of the regenerate or tumor, whereas that same degree of anoxia is evidently a factor protecting these tissues from the action of novoembichin and TET, just as has been shown by several workers in the case of ionizing radiation [1, 3, 5, 6, 14]. Our findings thus show that sarcolysin differs to a certain extent by its mechanism of action from novoembichin and TET; this probably depends on the presence of a natural group (phenylalanine) in the sarcolysin molecule. In the experiments with novoembichin and TET we were possibly confronted by a special phenomenon resembling the oxygen effect in radiobiology, and confirming the radiomimetic character of the action of these drugs on the cell. In contrast to novoembichin and TET, sarcolysin revealed distinctive features of its biological action that were not typical of radiomimetic substances and ionizing radiation.

SUMMARY

A comparative effect of the action of sarcolysin, novoembichin and TEM on the regeneration of the tadpole tail was studied both at the optimal and at subnormal temperatures (3-6° lower). Slowing of the metabolism provoked by the temperature reduction intensified the inhibitive action of sarcolysin on the tail regeneration, but weakened the effect of novoembichin and TEM. The author also investigated the action of sarcolysin and TEM on Ehrlich's tumor (transplanted into mice intramuscularly)-both on the usual strain and a strain subjected to prolonged chilling at 4°C. The inhibitive effect of sarcolysin on the tumor growth of the chilled strain was 3-4 times greater than on that of the normal strain. Conversely, the inhibitive effect of TEM on the growth of the normal strain tumors was double that exercised on the chilled tumors.

The combined effect of sarcolysin and chlorpromazine on a normal strain Ehrlich's tumor was also investigated. In this case the inhibitive effect of sarcolysin on the tumor was also intensified by the pharmacological hypothermia.

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