DIURNAL FLUCTUATIONS IN THE TOXICITY OF SARCOLYSIN

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The toxicity of sarcolysin as LD_{50} was studied in intact mice of different ages in relation to the time of its administration during the 24-hour period. Mortality among the animals showed two maxima, in the morning and late evening, and two minima, in the early evening and at night. The rats showed diurnal variations in incorporation of the radioactive label of sarcolysin-C^{14} into tissues sensitive to it: the spleen and mucous membrane of the small intestine. These fluctuations were similar in character to the diurnal fluctuations in the toxicity of sarcolysin.

Previous investigations have shown that the sensitivity of animals to antitumor drugs such as 5-fluorouracil, dopan, and olivomycin may vary during the 24-h period. At the same time it has been shown that the diurnal fluctuations in sensitivity to each of these substances have their own distinguishing features [4, 5].

A study of the literature shows that the intensity of cell division in normal animal tissues varies during the 24-h period. Since 5-fluorouracil, olivomycin, and dopan injure different systems of the body which differ in their diurnal pattern of proliferative activity, the writers postulated that the variations in the toxicity of these compounds depend on the mitotic pattern in the tissues sensitive to these substances.

The object of this investigation was to study diurnal variations in the toxicity of another widely used antitumor compound – sarcolysin (phenylalanine mustard). In addition, because of results showing that the distribution of compounds similar to sarcolysin is connected with that toxic effect [6], and because of observations showing a connection between the distribution of these substances in the body and mitotic activity in the corresponding tissues [8], it was decided to investigate the incorporation of sarcolysin-C^{14} into tissues sensitive to it at different times of day and night.

EXPERIMENTAL METHOD

The acute toxicity of sarcolysin was studied in nine experiments on intact mice (551 noninbred and 156 C57BL mice) of both sexes, kept and fed under identical conditions. In each experiment the animals were divided into 5-8 groups (with 9-24 mice in each group). Mice aged approximately 2-2.5 months were used in the first four experiments and mice aged 3-5 months in the next five experiments. Sarcolysin was injected intraperitoneally in a dose corresponding to LD_{50} (22-23 mg/kg) once a day into the animals of each group at 2, 5, 8, and 11 A.M. and 2, 8, and 11 P.M. From the results of these experiments the mortality among the animals in % was determined and statistical analysis was carried out using the χ^2 criterion. Observations on the animals continued for 1-1.5 months.

Since the principles of cell division in the tissues and organs of mice and rats are basically similar, and since LD_{50} of sarcolysin is almost identical for both animals, the incorporation of sarcolysin-C^{14} was studied in rats purely for convenience of the work. The 40 male rats with a mean weight of 140 g were divided into four groups with 10 animals in each group. Sarcolysin, with the β-carbon atom of the alanine residue tagged, and with a specific activity of 17 MCi/g, was injected as a single indicator dose of 2 mg/kg intra-

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TABLE 1. Specific Activity of Organs and Tissues after Administration of Sarcolysin-C^{14} at Different Times of Day or Night

Tissue or organ	Radioactivity of Organs and Tissues (in pulses/g wet weight of tissue in 100 sec) after administration of sarcolysin-C^{14}			
	8 A.M.	2 P.M.	5 P.M.	8 P.M.
Mucous membrane of small intestine	28 812±391	21 596±365	22 732±375	22 460±221
Spleen	2966±467	23 591±276	23 825±316	21 244±341
Bone marrow	11 038±308	10 875±341	12 115±244	11 195±244

peritoneally into the animals of group 1 at 7 A.M., and groups 2-4 at 2, 5, and 8 P.M. respectively. The rats were exsanguinated 15 min after the injection of the labeled compound, during the period of maximal radioactivity of the organs and tissues [7], and the spleen, bone marrow, and mucous membrane of the small intestine were removed. The extracted tissues were weighed and homogenized in five volumes ofphysiological saline. The radioactivity of 0.1 ml of the homogenate was determined on a gas-flow counter and calculated per gram wet tissue in pulses/100 sec. The Poisson distribution was used to determine the statistical error of measurement.

EXPERIMENTAL RESULTS

The results showing variations in the toxicity of sarcolysin varied with the time of its administration. Mortality of the animals was highest in all experiments after administration of the compound in the morning (5-11 A.M.). Mortality in some experiments reached a minimum at 8 P.M. and in others at 2-5 P.M. In the evening or night (8-11 P.M.) a second maximum of mortality was observed, and there was a second minimum at night.

In experiments No. 1-4 the lowest mortality among the mice was observed in groups receiving the compound at 8 P.M. and in experiments Nos. 5-9 in the groups receiving the compound at 2-5 P.M. In the older animals (experiments Nos. 5-9) the period of the morning and afternoon increase in sensitivity to sarcolysin was shortened, while the time of least sensitivity was correspondingly shifted from 8 P.M. to 2-5 P.M. In most cases the minimal toxic indices were significantly below the maximal by 5-7 times. Experiments Nos. 1 and 2, carried out simultaneously on mice of different sexes, showed that males and females react in the same way to injection of sarcolysin at different times of day or night. In C57BL mice (experiments Nos. 8, 9) the diurnal fluctuations in sensitivity to the compound were similar to those in noninbred animals.

It is clear from Table 1 that fluctuations in the intensity of incorporation of radioactive label (sarcolysin-C^{14}) into the mucous membranes of the small intestine and into the spleen of rats varied with the time of its administration. For instance, at 8 A.M. a higher concentration of radioactivity was observed in these tissues than at 2, 5, and 8 P.M. The difference in the specific activity of these tissues between the morning group and the other groups was significant. The radioactivity of the bone marrow was unchanged at all periods of investigation.

These diurnal fluctuations in the toxicity of sarcolysin were evidently connected with the rhythm of cell division in the tissues and organs of the animals. Proliferating cells are known to be more sensitive to the action of alkylating agents. Depression ofhematopoiesis and damage to the small intenstine are among the toxic manifestations of sarcolysin. The greatest damage by sarcolysin to tissues sensitive to it probably takes place in the period when their mitotic activity is maximal.

Most authors who have studied diurnal rhythms of cell reproduction in the intestine of mice and rats observed an increase in mitotic activity in the morning [1, 2, 3, 9]. It was at these times in the present series of experiments that the mortality among the animals was highest. The greatest concentration of sarcolysin-C^{14} in the mucous membrane of the small intestine and in the spleen also was observed after administration of the compound in the morning. It was accordingly postulated that the diurnal rhythm of cell division in these tissues is one of the factors which determines the diurnal rhythm of the sensitivity of animals to sarcolysin. The authors consider that the results, allowing for biological differences between tumors and normal tissues, provide a basis for research aimed at seeking the most rational program of sarcolysin administration.

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