STUDY OF REPAIR OF DNA—PROTEIN CROSS-LINKAGES INDUCED BY ANTITUMOR ALKYLATING AGENTS

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DNA—protein cross-linkages are a type of injury that has received little study. This is true both of induction of cross-linkages and of their repair, which has been discovered in recent years [4, 6, 8, 10, 11].

The object of this investigation was to study the formation and repair of DNA—protein cross-linkages, induced by three bifunctional alkylating agents, differing from one another in the "carriers" of the alkylating part of the molecule: the hydrochloride form of nitrogen mustard (embichin) and its derivatives — sarcolysin and chlorphenacyl, in which the di(2-chloroethyl)-amino group is attached to residues of β-phenyl-α-alanine and phenylacetic acid respectively. In this investigation an attempt was made to determine how changes in the chemical structure of the compound are reflected in its cross-linking ability. Besides the search for an effective cross-linking agent, the investigation also served another purpose. These compounds have strong cytostatic activity and are used in clinical oncology as antitumor agents. However, despite a long period of use, the mechanisms of their action have not been adequately studied. It is not yet known which molecular processes lie at the basis of the antitumor action of these compounds. DNA and proteins have been investigated as primary targets in this respect, but unambiguous results are still awaited [7].

EXPERIMENTAL METHOD

Experiments were carried out on Chinese hamster fibroblast-like cells cultured *in vitro*. The conditions of culture of the cells were described previously [4]. A 2-day culture grown in Carrel's flasks (seeding dose 20,000 cells/cm^2) in medium with ^{3}H-thymidine (1 μCi/ml medium, specific activity 5 Ci/mmole) was used. DNA—protein cross-linkages were induced by exposure of the cells for 1 h to the alkylating agents, after which they were incubated in medium without ^{3}H-thymidine. The method of determination of DNA—protein cross-linkages was described previously [4]. The number of cross-linkages was judged from the quantity of DNA (in percent) linked with protein after treatment of DNP, isolated from the cells, by uncoupling agents.

EXPERIMENTAL RESULTS

Embichin, sarcolysin, and chlorphenacyl were found to differ significantly in their ability to induce DNA—protein cross-linkages. Differences were found both in the concentration of the compounds required to produce cross-linkage and also in the kinetics of behavior of the cross-linkages they induced. To obtain comparable numbers of cross-linkages it was necessary to use sarcolysin and chlorphenacyl in concentrations 10 times higher than that of embichin. Embichin induces cross-linkages immediately after exposure. They are eliminated to the extent of 70-90% 24 h after washing out the agent. The cross-linkage elimination curve has been shown to be linear in character [9].

Sarcolysin was unable to induce cross-linkages immediately after exposure. An appreciable number of cross-linkages first appeared 4 h after rinsing out the preparation (Fig. 1). Their number increased after 24 h. Elimination of cross-linkages was not observed during the following day. This, however, does not mean that DNA—protein cross-linkages induced by sarcolysin do not, in principle, undergo repair. In the interval between 12 and 18 h in

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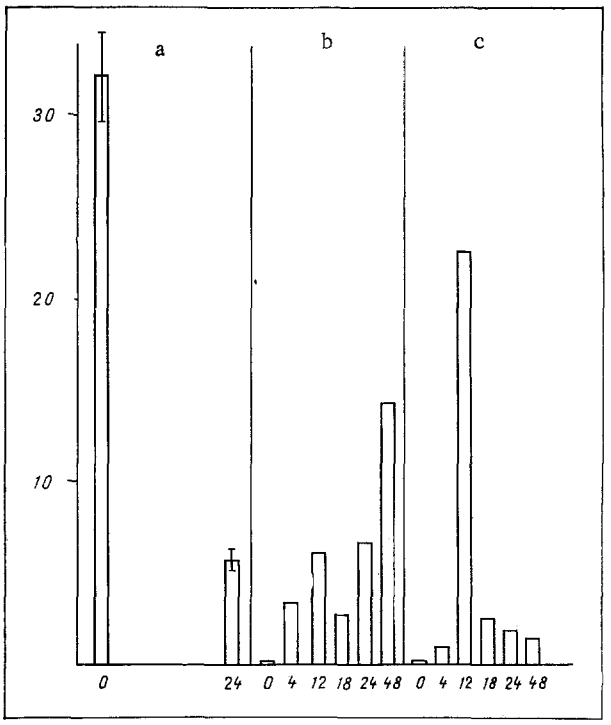

Fig. 1. Induction and repair of DNA—protein cross-linkages in culture of Chinese hamster cells during exposure to alkylating agents. Ordinate, % of DNA cross-linked with protein; abscissa, duration of incubation of cells after exposure for 1 h to agent followed by its rinsing out (in h). a) Embichin, 0.12 mmole (mean results of nine experiments); b) sarcolysin, 1.2 mmole (result of one typical experiment); c) chlorphenacyl, 1.5 mmole (result of one typical experiment).

TABLE 1. Induction and Repair of DNA-Protein Linkages in Culture of Chinese Hamster Cells during Exposure to Sarcolysin and Chlorphenacyl

Agent	Experiment No.	% of DNA cross-linked with protein at various times after treatment									
		0	4 h	7 h	9 h	12 h	15 h	18 h	21 h	24 h	48 h
Sarcolysin (1.2 mmole)	1	3,3	10,6	—	—	19,5	—	12,3	—	25,0	—
	2	0	9,1	—	—	27,8	—	25,4	—	47,6	—
	3	0	3,2	—	—	5,9	—	2,6	—	6,5	14,2
	4	1,1	4,6	—	—	3,7	—	4,7	—	12,4	8,2
	5	1,3	18,3	—	—	9,1	—	24,0	—	16,4	14,6
	6	—	10,7	15,2	15,7	13,1	12,7	21,3	20,6	20,0	—
	7	—	5,9	8,2	4,1	4,0	0,9	3,0	2,8	7,0	—
Chlorphenacyl (1.5 mmole)	1	3,7	3,1	—	—	15,6	—	3,7	—	5,5	—
	2	1,2	1,2	—	—	11,2	—	2,8	—	0	—
	3	0	0,8	—	—	22,6	—	2,2	—	1,7	1,3
	4	0,8	1,8	—	—	3,8	—	4,8	—	2,0	—
	5	0	1,9	—	—	6,1	—	3,3	—	1,8	0,7

Legend. Values given after deduction of control.

all experiments the number of cross-linkages fell on average by 33% (Table 1). This was confirmed by the results of experiments in which cross-linkages were determined at intervals of 2-3 h, and not of 4 h (Table 1). Under these circumstances a decrease in the number of cross-linkages within the specified time interval was observed not at one, but at two or three points.

Distinguishing features of the kinetics of behavior of sarcolysin-induced cross-linkages must be taken as evident that in this case two types of cross-linkages are formed — "early," reparable cross-linkages and "late" cross-linkages, reparable only with difficulty or not at all. As regards the character of its cross-linking action, sarcolysin differs from embichin in being a compound with delayed and prolonged action.

Chlorphenacyl gave a different picture. Like sarcolysin, it induced cross-linkages 4 h after exposure (Fig. 1), but their number increased significantly until 12-18 h, after which it fell, as a rule considerably. With respect to reparability, cross-linkages induced by chlorphenacyl were similar to those induced by embichin, but they appeared more slowly. Consequently, chlorphenacyl is a compound with delayed but not prolonged action.

The kinetics of behavior of cross-linkages was compared with the therapeutic efficacy of the compounds. One indicator of activity of the preparation is what is called the chemotherapeutic index, i.e., the ratio of its toxicity to its antitumor action. This index is calculated as the ratio between the single dose causing death of 50% of the animals to the daily dose causing inhibition of growth of the tumor by 50% [3]. Embichin had the lowest chemotherapeutic index, namely 1.1 [2]. In its action embichin is an effective, but very toxic preparation, and for that reason it has been withdrawn from production in the Soviet Union and replaced by less toxic derivatives. The chemotherapeutic index of chlorphenacyl is 27.9 [1]. This compound is less toxic than embichin. Various derivatives of chlorphenacyl have recently found application in chemotherapy. Sarcolysin has a chemotherapeutic index of 60-71 [1, 2]. This compound has the mildest action, yet at the same time it is a very active drug, widely used in tumor chemotherapy. It is evident that the therapeutic activity of the three compounds studied can be correlated with the kinetics of behavior of the DNA—protein cross-linkages. An essential factor in the efficacy of a preparation is evidently delay of the appearance of cross-linkages and their poor reparability, as is clear from the example of sarcolysin. A further study of relations between the therapeutic action of the compound and its ability to form DNA—protein cross-linkages will help to reveal whether the correlation discovered in these experiments is accidental or a regular phenomenon.

The results are evidence that introduction of organic residues into the nitrogen mustard molecule sharply modifies not only the antitumor and alkylating activity of the compounds, but also their cross-linkage characteristics. Delayed cross-linkage formation, characteristic of sarcolysin and chlorphenacyl, is interesting. Since the active ethylenimmonium ion, which performs the alkylation, in aromatic analogs of nitrogen mustard is extremely unstable (its half-hydrolysis period in aqueous solution is about 30 min) [5], it can be tentatively suggested that delayed cross-linkage (after 4 h or more) takes place on account of long-lived injuries to DNA and protein, by contrast with cross-linkages formed by short-lived injuries from embichin. The fact of delayed appearance of the cross-linkages indicates that when a particular preparation is being tested for cross-linking activity, the kinetics of appearance of the cross-linkages with time must be studied, for the initial periods after exposure to the compound may not give a true idea of its cross-linking ability.

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