EFFECT OF VARIOUS PROCEDURES ON NUCLEOSIDE PHOSPHATE KINASE ACTIVITY OF NORMAL AND TUMOR CELLS

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Despite the ability of most organisms to synthesize ribo- and deoxyribonucleotides *de novo*, in many of their cells nucleotide formation also takes place by direct phosphorylation of purine and pyrimidine nucleosides. The enzymes of this shunt, nucleoside phosphate kinases, may play an important role in the activity of some fast-growing normal animal and human tissues, such as, for example, bone marrow, regenerating liver, and neoplastic tissue. The level of nucleoside phosphate kinase activity in the cells of neoplasms may be one factor determining the sensitivity of tumors to the antitumor action of antimetabolites belonging to the group of purine or pyrimidine derivatives [1].

The aim of this investigation was to study the effect of various procedures on nucleoside phosphate kinase activity in human and animal tumors and in the liver of tumor-free animals.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats and mice, male SHR rats, and samples of human lung tumors (squamous-cell carcinoma) obtained at operation. In experiments in which tumor-bearing animals were treated with antitumor compounds 63 rats and 110 mice were used. The substances were injected into the experimental animals in therapeutic doses for 5 days. The following antitumor agents were used: cyclophosphamide, 5-fluorouracil, prospidine*, dioxadet, and also compounds exhibiting some antitumor activity under experimental conditions, namely derivatives of chloroaminodioxan (No. 115) and disiloxane (No. 216). Only those lung specimens were studied in which inhibition of growth as a result of the action of the agent was not less than 50%.

In experiments to study induction of liver tumors by nitrosoethylamine (NEA) or 2-acetylaminofluorene (AAF) 60 rats were used (20 animals in a group). The experimental animals were given NEA with their drinking water (0.01% solution) or AAF through a tube in a dose of 4 mg per animal 5 times a week, in the form of a suspension in agar-agar gel. The animals were decapitated 2 months after the beginning of NEA or 3 months after the beginning of AAF administration. The liver was studied histologically and only the tumor tissue was subjected to biological analysis.

Pieces of human lung tumors were obtained from 18 unirradiated patients and from 3 patients who had undergone preoperative irradiation.

Cell-free tissue extracts were obtained as described previously [5]. The supernatant obtained after ultracentrifugation of the homogenates was dialyzed against 5 mM Tris-HCl, pH 7.4, and used as the source of phosphorylating enzymes. Activity of uridine and thymidine phosphate kinases was determined in a reaction mixture with a volume of 220 µl, containing 20 µl of cell free extract (120-160 µg protein), 40 µmoles Tris-HCl, 1 µmole ATP, 2.5 µmoles $MgCl_2$, 2 µmoles uridine or thymidine, and also 2 pmoles each of the corresponding

*N,N"'-di-(γ-chloro-β-hydroxypropyl)-N,N"'-dispirotripiperazine dichloride.

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TABLE 1. Action of Compounds with Antitumor Activity, of X-Ray Irradiation, and of Carcinogens on Thymidine Phosphate Kinase and Uridine Phosphate Kinase Activity (in percent of control taken as 100)

Test object	Antitumor factor	Enzyme	
		Thymidine phosphate kinase	Uridine phosphate kinase
Mouse sarcoma 180	5-fluorouracil	26*	174*
	Cyclophosphamide	92	17*
	Prospidine	57*	128
	No. 115	35	—
Pliss rat lymphosarcoma	Dioxadet	22*	127*
Mouse carcinoma 755	No. 216	523*	101
Human squamous-cell lung carcinoma	Irradiation	0*	500*
Intact rat liver	AAF	17*	220*
	NEA	60*	180*

Legend. Each number is the mean of 2 or 3 parallel experiments carried out in 7 animals. In experiments on human lung tumors each sample of tumor was analyzed separately. *$P < 0.05$ compared with control.

^{3}H-nucleoside. The samples were incubated for 1 h at 37°C. The reaction was stopped by heating the samples in a boiling water bath for 30 sec and the residue was separated by centrifugation at 3,000g for 10 min. Supernatant from each sample, in a volume of 10 µl, was applied to DEAE-cellulose plates (9 × 12 cm) and subjected to ascending chromatography in 0.04 M HCl. Unlabeled TMP or UMP was used as reference substance. Zones corresponding to the location of the monophosphates were identified by means of an ultrachemiscope, cut out with a scalpel, and transferred to scintillator. Radioactivity was determined with a Mark II liquid scintillation counter and expressed in picomoles of converted substrate per milligram protein per hour of incubation. Protein was determined by the microbiuret method [6]. The results were subjected to statistical analysis by the Wilcoxon–Mann–Whitney U test [3] and the degree of correlation was calculated by Spearman's equation [4].

EXPERIMENTAL RESULTS

The results obtained in experiments on transplanted tumors in mice and rats treated with antitumor compounds, on human lung tumors after x-ray irradiation, and on rat liver tumors induced by hepatocarcinogens, are given in Table 1. The factors used, despite their different nature, were found to inhibit TMP formation from thymidine equally (experiments to study the reaction of compound No. 216 on mice with carcinoma 755, in which the rate of TMP synthesis was increased fivefold, were exceptions). These same procedures caused an increase in the rate of UMP synthesis from uridine (except in experiments in which mice with sarcoma 180 were treated with cyclophosphamide). In fact, under the influence of, for example, prospidine or 5-fluorouracil, activity of thymidine phosphate kinase in mice with transplanted sarcoma 180 was reduced whereas uridine phosphate kinase activity was increased. After preoperative irradiation of the lungs, uridine phosphate kinase activity increased whereas TMP synthesis from thymidine was virtually absent. Under the influence of AAF on intact rats the thymidine phosphate kinase activity of the hepatocytes was reduced whereas phosphorylation of uridine increased. On the whole negative correlation was observed between changes in UMP and TMP synthesis ($r = -0.86$, $n = 8$, differences significant at the $P \leqslant 0.05$ level).

The results thus indicate opposite changes in activity of enzymes of TMP and UMP synthesis as a result of the action of antitumor agents of different

We know that the thymidine kinase pathway of TMP biosynthesis is "spare" and plays an essential role in DNA synthesis when the thymidylate synthetase pathway is blocked. For example, the action of antifolate preparations, with both teratogenic and antitumor activity, leads to blocking of the thymidylate synthetase pathway: Under these circumstances thymidine kinase activity is increased [2].

The results suggest the existence of yet another mechanism of compensation: a thymidine kinase — uridine kinase shunt, which can maintain the necessary intracellular level of UMP and TMP concentrations through interconversions of thymidine and uridine during methylation (demethylation) and also reduction (oxidation) of ribose. This shunt can play a definite role when cell homeostasis is disturbed as a result of the action of compounds with antitumor or carcinogenic activity or of x-ray irradiation.

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