Role of Deoxyribonuclease in Cancer Chemotherapy

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Abstract □ It has been postulated that the lysosomal enzyme deoxyribonuclease is responsible for nicks in both strands of deoxyribonucleic acid so that alkylating agents can cross-link the nucleotides of the two strands. Administration of an alkylating agent, *p*-di(2-chloroethyl)amino-L-phenylamine, to CF_1 mice results in elevated activities of free deoxyribonuclease in Ehrlich ascites tumor cells and normal liver homogenates and isolated lysosomal fractions. The drug acts specifically on the release of the hydrolytic enzyme deoxyribonuclease rather than nonspecific rupture of the lysosomal membrane. Deoxyribonuclease activity increases in cancer tissue as cell proliferation continues.

Keyphrases □ Deoxyribonuclease—role in cancer chemotherapy, effect of *p*-di(2-chloroethyl)amino-L-phenylamine on activity □ Cancer chemotherapy—role of deoxyribonuclease, effect of alkylating agent on activity □ *p*-Di(2-chloroethyl)amino-L-phenylamine—effect on deoxyribonuclease activity

The nitrogen mustard *p*-di(2-chloroethyl)amino-L-phenylamine[1] (I) is a phenylalanine derivative. Compound I is used in the treatment of multiple myeloma (1) and Burkitt's lymphoma (2). The accepted mechanism of action of a nitrogen mustard containing two functional centers is the cross-linking of twin strands of deoxyribonucleic acid at the 7-position of guanine and, to a lesser extent, at the 3- and 1-positions of adenine (3). This prevents deoxyribonucleic acid replication, and cell proliferation is halted probably in the S or G_2 period of the cell cycle of tumor cells (4). Furthermore, labilization of the alkylated guanine occurs with the formation of apurinic acid, which is unstable and decomposes with the breakage of the backbone of the deoxyribonucleic acid strand (5). For the nitrogen mustard to induce cross-linking in the nucleotide strands, the nucleotide strands must be broken initially. Allison (6) reported that all cells have substantial amounts of deoxyribonuclease in the lysosomes and that this enzyme is capable of breaking both strands of a double helix of the nucleic acids. Consequently, this study was designed to determine if Compound I treatment results in elevated deoxyribonuclease activity in normal and cancerous tissue.

EXPERIMENTAL

Maintenance of Tumor—CF_1 male mice were transplanted intraperitoneally with 0.1 ml of 7-day Ehrlich ascites tumor cells from infected mice. The average life expectancy for these mice was 14.9 days after injection. Transplantation was normally carried out once a week to maintain the tumor cell line in these laboratories.

Cytotoxicity of Compound I—A single dose (20 mg/kg) of Compound I in 1% carboxymethylcellulose was administered orally on Day 3 or 7 after tumor transplant. Twenty hours after dosing, the animals were sacrificed by cervical dislocation and the ascites tumor was drained from the peritoneal cavity. A microcrit and the total volume of the tumor were recorded for each animal.

Table I—Percent Free Deoxyribonuclease Activity of Ascites Tumor Cells

	N	$\bar{x} \pm SD$, %	p
Day 4 control	8	13 ± 5	—
20 mg/kg I	8	23 ± 2	0.001
Day 8 control	18	33 ± 3	—
20 mg/kg I	18	37 ± 4	—

Table II—Percent Free Deoxyribonuclease Activity of CF_1 Mouse Liver Homogenate

	N	$\bar{x} \pm SD$, %	p
Control	19	19 ± 3	—
I (*in vivo*):			
1 hr after 1 mg	12	28 ± 6	0.001
20 hr after 5 mg	8	24 ± 6	0.010
20 hr after 10 mg	18	26 ± 5	0.001
20 hr after 20 mg	12	23 ± 5	0.010

Free and Total Deoxyribonuclease Activity—*In Vivo*—Free and total acid deoxyribonuclease activity was determined according to the method of deDuve *et al.* (7). The detergent octoxynol[2] (0.2%) was used to release all bound lysosomal enzyme. Deoxyribonuclease was determined on the livers of normal animals and on the livers of animals treated with 20 mg/kg of I 20 hr previously. Deoxyribonuclease activity was also determined on ascites tumor fluid on Day 4 or 8 for controls and on animals 20 hr after treatment with 20 mg/kg of Compound I.

Free and total acid phosphatase activity of Ehrlich ascites tumor and mouse liver was determined by the Gianetto and deDuve method (8). The inorganic phosphate released was measured by the method of Chen *et al.* (9).

In Vitro—Free and total deoxyribonuclease activity was determined on liver after incubating at 37° for 1 hr in a metabolic shaker[3], using 1 ml of a 20% homogenate in 0.25 *M* sucrose + 0.001 *M* ethylenedinitrilotetraacetic acid (pH 7.2) and 5 ml of Hanke's basic salt medium reinforced with glucose (pH 7.2). Zero, 0.5, 1, and 2 mg of I were added to the medium.

Lysosomal Preparation—A highly purified liver lysosomal fraction was obtained from normal mice and from mice 20 hr after treatment with 20 mg/kg of I using the method of Sawant *et al.* (10). Fraction IV (17,000×*g* for 20 min) was used to determine the deoxyribonuclease enzymatic activity of purified lysosomes.

Statistical Analysis—Tables I-V list the number of animals in the group, expressed as *N*, and the mean and standard deviation, expressed as $\bar{x} \pm SD$. The probable significant level *(p)* between each test group and the control group was determined by the Student *t* test (11).

RESULTS

Compound I caused a 96% inhibition (N = 10) of ascites tumor growth of mice administered the drug on Day 3 and a 71% inhibition when administered on Day 7. The deoxyribonuclease activity of Ehrlich ascites tumor fluid increased significantly from Day 4 to Day 8. Compound I treatment at 20 mg/kg ip resulted in an accelerated deoxyribonuclease activity above that of the normal cancer cell on the given day examined (Table I). Treatment with Compound I also resulted in elevation in mouse liver deoxyribonuclease activity both *in vivo* and *in vitro* (Tables II and III). *In vitro,* high concentrations of the drug inhibited the *free deoxy-*

[1] Alkeran brand Melphalan, Burroughs Wellcome Co.

[2] Triton X-100, Rohm & Haas Co.
[3] Dubnoff.

Table III—*In Vitro* 1 hr Percent Free Incubation Deoxyribonuclease Activity of CF_1 Mouse Liver Homogenate

	N	$\bar{x} \pm SD$, %	p
Control	19	31 ± 4	—
0.5 mg I	8	44 ± 12	0.001
1.0 mg I	10	19 ± 4	0.001
2.0 mg I	5	17 ± 9	0.001

Table IV—Deoxyribonuclease Activity of Lysosomes Isolated from CF_1 Mouse Liver

	N	$\bar{x} \pm SD$, %	p
1% carboxymethylcellulose, control	8	52 ± 3	—
20 mg/kg I	8	60 ± 6	0.01

ribonuclease activity. When the lysosomes were isolated from normal and treated liver, it was observed that treatment with Compound I resulted in a significant elevation of lysosomal hydrolytic deoxyribonuclease activity of the lysosomal preparation (Table IV). Acid phosphatase is a well-known hydrolytic lysosomal enzyme; when its activity was measured in the liver of mice treated with I, no increase in activity was noticed but an inhibition of enzymatic activity was observed. The total lysosomal activity of deoxyribonuclease and acid phosphatase was unaltered by treatment with I.

DISCUSSION

Compound I causes an increase in percent free acid deoxyribonuclease activity of liver and Ehrlich tumor cells. The deoxyribonuclease hydrolytic enzyme is released into the cell sap by the drug from the lysosomes, where it is normally bound and inert. Purified lysosomal deoxyribonuclease can cause breaks in chromatin material (6). Davidson (12) claimed that nucleases are probably involved in maturation of ribonucleic acid, in the synthesis of deoxyribonucleic acid, and in the recombination events of proliferating cells. According to the Kornberg (13) theory, replication begins with the introduction of a nick in one strand, possibly by the action of an endonuclease. Deoxyribonucleic acid polymerase then binds to the nick and begins to extend the 3′-hydroxyl end of the nicked strand. The endonuclease can also cleave the newly formed strands at forks, resulting in short segments of the nucleic acid which are ligated together enzymatically. Thus, it is not surprising that a 2.5-fold increase of deoxyribonuclease activity was observed in the Ehrlich tumor cells as the cells proliferated over 8 days.

This study supports the idea that chemotherapy drugs, once introduced into the cell, react with lysosomal hydrolases to cause their release in cancer cells and in normal cells and direct the drug to its cellular site of action, *i.e.*, the nucleic acid (14–16). The action of I is specific for the enzyme deoxyribonuclease, which is needed for the cross-linking of the alkylating drug in the deoxyribonucleic acid strands. Since the total lysosomal enzymatic activity of acid phosphatase and deoxyribonuclease was normal after treatment with I, the drug did not lyze the lysosomal membrane as a detergent would. Furthermore, treatment with I does not cause an increase in free activity of the enzyme acid phosphatase, a known marker of hydrolytic activity. Thus, it appears that the action of I on lysosomes is specific for the release

Table V—Percent Free Acid Phosphatase Activity of CF_1 Mouse Liver Homogenate

	N	$\bar{x} \pm SD$, %	p
Control	20	59 ± 7	—
I (*in vivo*):			
1 hr after 1 mg	12	4 ± 4	0.001
2.5 hr after 1 mg	8	2 ± 2	0.001
20 hr after 20 mg	16	13 ± 11	0.001

of deoxyribonuclease and does not involve a general rupture of the lysosomal membrane. Since alkylating agents are capable of electrophilic reactions with functional groups of proteins (*i.e.*, ionized thiol, amine, ionized phosphate, and ionized carboxylic acid), it can be postulated that the polypeptide chains of the deoxyribonuclease enzyme are attached and then the enzyme acts as a carrier of the drug to the nucleus, resulting in cross-linking of the deoxyribonucleic acid strands.

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