POTENTIATION OF THE ACTIVITY OF ANTITUMOR AGENTS BY DIURETICS

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The effect of diuretics (acetazolamide, chlormerodrin, frusemide, hypothiazide, and acetazolamide alternately with chlormerodrin) on the antitumor activity of dipin,* cyclophosphamide, 6-mercaptopurine, and serotonin against sarcoma 45 of rats was studied. In most experiments the diuretics potentiated the antitumor action of the compounds. Acetazolamide was more effective when given simultaneously with the cytostatics, chlormerodrin was more effective when given 3-4 h after the antitumor agent, and hypothiazide if given 5-6 days before the basic treatment. The toxicity of the cytostatic drugs was virtually unaffected by combination with the diuretics.

*Tetraethyleneimido-piperazine-N,N'-diphosphoric acid.

TABLE 1. Effect of Diuretics on Activity of Antitumor Agents when Given in Different Orders of Precedence

Variant of expt.	No. of anim.	Treatment scheme	Wt. of tumor(g)	P	Inhibition (%)
Control	6	—	29,0±2,1	—	—
Phenylalanine mustard	6	—	21,3±2,5	<0,05[1]	27
Hypothiazide + phenylalanine mustard	6	Consecutively	7,8±2,9	<0,05[1] <0,05[2]	73
Control	7		18,5±2,6	—	—
Phenylalanine mustard	10		11,2±1,5	<0,05[1]	39
Phenylalanine + hypothiazide	10	Simultaneously	12,0±2,2	<0,05[1] >0,05[2]	35
Control	7		11,9±1,2	—	—
Phenylalanine mustard	6		6,8±1,1	<0,05[1]	43
Acetazolamide + phenylalanine mustard	6	Consecutively	6,5±0,9	<0,05[1] >0,05[2]	46
Control	6		19,2±1,0	—	—
Phenylalanine mustard	6		12,8±2,1	<0,05[1]	34
Phenylalanine + acetazolamide	6	Simultaneously	4,2±1,4	<0,05[1] <0,05[2]	78
Control	7		21,4±1,2	—	—
Phenylalanine mustard	6		9,3±1,3	<0,05[1]	57
Chlormerodrin + hypothiazide	7	Consecutively	9,1±0,8	<0,05[1] >0,05[2]	58
Control	6		19,2±1,0	—	—
Phenylalanine mustard	6		12,8±2,1	<0,05[1]	34
Phenylalanine mustard + chlormerodrin	6	Simultaneously	3,6±1,1	<0,05[1] <0,05[2]	81

[1]Here and in Table 2, comparison with control.
[2]Here and in Table 2, comparison with group of animals receiving antitumor agent only.

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TABLE 2. Effect of Diuretics on Activity of Antitumor Agents when Given in Different Orders of Precedence

Variant of expt.	No. of anim.	Frusemide	Wt. of tumor(g)	P	Inhibition (%)
Control	6	—	43,8±4,8	—	—
Phenylalanine mustard	7		25,8±4,2	<0,05[1]	41
Phenylalanine mustard + acetazolamide	7	Simultaneously	12,3±1,4	<0,05 [1] <0,05[2]	72
Phenylalanine mustard + acetazolamide	7	3-4 h	18,9±3,0	<0,05[1] >0,05[2]	57
Acetazolamide + phenylalanine mustard	7	3-4 h	15,7±2,2	<0,05[1] <0,05[2]	64
Dipin	6		15,5±1,7	<0,05[1]	65
Dipin + chlormerodrin	6	Simultaneously	19,7±2,7	<0,05[1] >0,05[2]	56
Dipin + chlormerodrin	7	3-4 h	10,8±0,3	<0,05[1] <0,05[2]	76
Chlormerodrin + dipin	6	3-4 h	18,5±2,7	<0,05[1] >0,05[2]	58
Control	6		15,8±3,0	—	—
Dipin	6		6,5±1,6	<0,05[1]	59
Dipin + acetazolamide	6	Simultaneously	0,4±0,2	<0,05[1] <0,05[2]	98
Dipin + acetazolamide	6	3-4 h	1,1±0,3	<0,05[1] <0,05[2]	93
Acetazolamide + Dipin	6	3-4 h	0,83±0,1	<0,05[1] <0,05[2]	95
Control	6		16,0±3,0	—	—
Phenylalanine mustard	6		9,2±2,4	<0,05[1]	42
Phenylalanine mustard + chlormerodrin	6	Simultaneously	6,3±3,7	<0,05[1] >0,05[2]	57
Phenylalanine mustard + chlormerodrin	6	3-4 h	4,8±1,1	<0,05[1] <0,05[2]	70
Chlormerodrin + phenylalanine mustard	6	3-4 h	7,8±1,9	<0,05[1] >0,05[2]	51

TABLE 3. Blood Leukocyte Count of Rats with Sarcoma 45 after Treatment for 10 Days with Cytostatics and Diuretics

Variant of experiment	No. of anim.	Leukocyte count	Wt. of tumor(g)	Inhibition (%)
Control	—	26 350 (2)	30,0±3,7 (10)	—
Dipin	10	15 700 (2)	8,5±2,6 (10)	71,5
"	15	6 150 (2)	4,85±1,3 (10)	84,0
Dipin + acetazolamide	10 200	19 000 (2)	2,67±0,81 (10)	91,0
Control	—	37 360±4 950 (4)	14,6±2,7 (10)	—
Cyclophosphamide	8	12 250±3 450 (4)	3,75±0,9 (10)	74,5
"	12	7 300±1 640 (4)	0,75±0,47 (10)	95,0
Cyclophosphamide + acetazolamide	8 200	14 430±2 400 (4)	1,88±0,47 (10)	85,6
Control	—	39 500±9 900 (4)	24,6±1,6 (10)	—
Phenylalanine mustard	1,0	17 840±3 250 (4)	13,0±2,2 (10)	47,1
" "	1,2	12 150±2 300 (4)	10,8±0,83 (10)	56,6
Phenylalanine mustard + acetazolamide	1,0 200	17 440±2 700 (4)	7,0±1,9 (10)	72,0
Phenylalanine mustard + chlormerodrin	1,0 18	16 980±5 700 (4)	8,3±1,7 (10)	66,2
Control	—	25 640±6 600 (4)	8,3±1,5 (10)	—
Dipin	10	3 525±720 (4)	0,33±0,14 (10)	96,0
"	15	1 840±85 (4)	0,01±0,005 (10)	99,9
Dipin + acetazolamide	10 200	3 180±225 (4)	0,01±0,008 (10)	99,9
Dipin + chlormerodrin	10 18	3 390±206 (4)	0,095±0,08 (10)	98,9
Dipin + frusemide	10 50	3 840±700 (4)	0,086±0,024 (10)	99,0

Note: Number of animals in experiment given in parentheses.

The growth of experimental malignant neoplasms is often accompanied by retention of water and salts in the body [2, 3, 5]. Effective anti-tumor therapy sometimes restores the normal water and salt balance [1, 4, 5].

This has led to the suggestion that stimulation of the elimination of water and salts from the body will facilitate the treatment of tumors. To test this hypothesis certain diuretics were used in conjunction with antitumor agents for the treatment of rats with sarcoma 45.

EXPERIMENTAL METHOD AND RESULTS

Experiments were carried out on 1500 rats with sarcoma 45. Treatment with antitumor agents began on the 7th day after transplantation of the tumor. Phenylalanine mustard, cyclophosphamide, and 6-mercaptopurine were injected intraperitoneally in doses of 1, 8, and 25 mg/kg, respectively, while dipin and serotonin were injected subcutaneously in a dose of 10 mg/kg daily for 10 days. The diuretics (acetazolamide, 200 mg/kg; chlormerodrin, 18 mg/kg; frusemide and hypothiazide, 50 mg/kg) were given by gastric tube as a starch suspension in various combinations with the antitumor agents. During the period of the experiments the animals were kept under constant conditions.

Investigations have shown that the isolated administration of diuretics by a 10-day course has no effect on the growth of sarcoma 45 of rats. Only by the alternate administration of acetazolamide with chlormerodrin (acetazolamide on one day, chlormerodrin on the next throughout the course of treatment) in some experiments led to some degree of inhibition of growth of the tumor (20-30%). The weight of the spleen, the increase in body weight, and the blood leukocyte count remained virtually unchanged. The combined administration of diuretics with antitumor agents in most cases increased the antitumor activity of the latter compounds. An important factor was the order of administration of the diuretics and antitumor agents (Tables 1 and 2). The order of treatment was as follows: on the 2nd-3rd day after transplantation of the tumor, treatment with the diuretic alone began, but from the 7th-8th day the diuretic was stopped and phenylalanine mustard was given for 10 days. As Table 1 shows, the preliminary (5-6 days before the beginning of cytostatic treatment) injection of hypothiazide gave a substantial increase in antitumor activity, by contrast with the simultaneous administration of the two agents. For acetazolamide and chlormerodrin, their simultaneous administration with phenylalanine mustard is more rational.

From acetazolamide and chlormerodrin other patterns of administration of the drugs were studied: simultaneously with the cytostatics of at intervals of 3-4 h (antitumor agent first, diuretic 3-4 h later, and vice versa). As Table 2 shows, acetazolamide had the maximal potentiating effect on the action of phenylalanine mustard and dipin was obtained by simultaneous administration with the cytostatics. In the case of acetazolamide the optimal variant of the experiment was that in which the diuretic was given 3-4 h after dipin or phenylalanine mustard.

The results showed that diuretics can potentiate the effect of antitumor agents (Table 3). It is important to find out how the toxicity of antitumor agents is effected by their combination with diuretics. The experimental results showed that diuretics did not change the blood leukocyte count by comparison with that in animals receiving the antitumor agents alone. An increase in the dose of cytostatic compounds was accompanied by a more marked inhibitory action of these substances on leukopoiesis. Achievement of the same antitumor effect by giving a smaller dose of the cytostatic in conjunction with diuretics did not change this index.

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