ONCOLOGY

Modification of Hemostatic Status Improves Antitumor Efficiency of Photodynamic Therapy

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Complex treatment with acetylsalicylic acid, heparin, and dexamethasone improves the efficiency of photodynamic therapy in rats with myosarcoma-I.

Key Words: *photodynamic therapy of tumors; hemostasis system*

The hemostasis system is involved in the pathogenesis and metastasizing of tumors. Anticoagulant-disaggregation correction of the hemostasis system improves the antitumor efficiency of radio- and chemotherapy [8].

Photodynamic therapy (PDT) is a perspective methods for the treatment of tumors due to its high efficiency and low incidence of complications. PDT is based on more rapid absorption of photosensitizer by intensively proliferating cells and generation of cytotoxic singlet oxygen during exposure to light. Positive effect of PDT can be due not only to direct damage to tumor cells, but also damage to blood vessels, induction of thromboses and hemorrhages in the tumor [7].

We studied the possibility of modifying the antitumor effect of PDT by correcting the hemostasis system.

MATERIALS AND METHODS

Experiments were carried out on female Wistar (n=65) and random-bred (n=30) rats weighing 140-180 g. Myosarcoma-I (M-I, 1-mm^3 fragments) was transplanted intramuscularly into the thigh. PDT was carried out 10-12 days after tumor transplantation, when its size reached 0.8-1.0 cm^3. Photosensitizer photosense was injected intraperitoneally in a dose of 5 mg/kg. Photoexposure (ATO-150 lamp, l=670 nm, total dose 300 J) was carried out 24 h after injection of photosense. Tumor size was measured before and on days 3, 7, 10, 14, and 21 after treatment. Aspirin (10 mg/kg, orally) *per os*, dexamethasone (0.63 mg/kg, orally) and heparin (400 U/kg, subcutaneously) were administered once a day for 5 and 10 days starting from the next day after PDT session. The choice of drugs and their doses was based on published reports on their effects on the hemostasis parameters and antitumor effect.

For evaluation of the hemostasis parameters, blood was collected from the abdominal aorta under Nembutal narcosis (35 mg/kg) and stabilized with 3.8% sodium citrate (9:1); for evaluation of platelet count the blood was collected from the caudal vein.

Recalcification time, prothrombin and thrombin time, fibrinogen concentration and activity of factor XIII, spontaneous fibrinolysis and ADP-induced platelet aggregation were evaluated [1]. Platelets were counted was described elsewhere[4], antiaggregation activity of the vascular wall was determined [10].

The results were processed statistically using Student's t test.

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TABLE 1. Effect of Complex Treatment with Aspirin, Heparin, and Dexamethasone for 5 Days on Hemostasis Parameters in Control Rats on Day 5 of Therapy (*M*±*m*)

Parameter	Control	Experiment
Recalcification time, sec	127.0±3.0 (13)	600*
Prothrombin time, sec	21.9±0.2 (13)	27.5±0.4* (16)
Thrombin time, sec	18.6±0.3 (13)	600*
Fibrinogen concentration, g/liter	1.4±0.3 (13)	0.7±0.5* (16)
Factor XIII activity, sec	26.1±2.7 (11)	13.0±1.2* (18)
Spontaneous fibrinolysis, %	12.5±1.6 (12)	51.8±4.1 (14)
Platelet count, 10^9/liter	641.0±23.8 (9)	612.0±32.4 (10)
Platelet aggregation, %	32.0±1.8 (7)	35.0±1.4 (6)
Antiaggregation activity of vascular wall, %	57.0±3.8 (5)	14.0±2.1* (7)

Note. Here and in Table 2: *$p<0.05$ compared to the control. Number of animals is given in parentheses.

TABLE 2. Size of M-I Tumor (cm^3) in Rats after PDT and Therapy with Aspirin, Heparin, and Dexamethasone (AHD) (*M*±*m*)

Term of investigation		PDT	PDT+AHD	
			5 days	10 days
Before PDT		0.34±0.14	0.31±0.05	0.23±0.04
After PDT, day	3	0.12±0.12	0.16±0.06	0.10±0.04
	7	0.98±0.27	0.52±0.20	0.30±0.09*
	10	2.12±0.56	1.58±0.84	0.90±0.14
	14	4.01±0.83	2.99±0.56	1.45±0.24*
	21	11.33±2.85	3.63±0.90*	4.50±0.80*

RESULTS

In control rats, treatment with aspirin, heparin, and dexamethasone led to a pronounced anticoagulant shift (Table 1). The decrease of vascular wall antiaggregation activity corresponded to aspirin dose, but the expected decrease of platelet aggregation intensity was not achieved, probably due to the fact that heparin in high doses stimulates platelet aggregation [2].

Three days after PDT session, the tumor decreased in size by more than 2-times (Table 2). Complex therapy decelerated tumor growth in subsequent terms. The most pronounced effect was observed when aspirin, heparin, and dexamethasone were administered for 10 days after PDT.

Complex therapy did not inhibited platelet aggregation and decreased vascular wall antiaggregation activity, and hence, its antitumor effect cannot be attributed to inhibition of the vessel-platelet hemostasis [9]. A more plausible explanation is blockade of prostaglandin (PG) synthesis with aspirin: PGE_2 stimulating tumor cell proliferation and PGF_2 stimulating inflammatory reaction [5].

Heparin potentiates the inhibitory effect of glucocorticoids on neoangiogenesis [3]. Moreover, hypocoagulation shift in the hemostasis system can also play a role in potentiation of the photodynamic antitumor effect.

Hence, modification of hemostasis system stimulates the antitumor effect of PDT.

REFERENCES

1. V. P. Baluda, Z. S. Barkagan, E. D. Gol'dberg, *et al., Laboratory Methods of Investigating the Hemostasis System*, Ed. E. D. Gol'dberg [in Russian], Tomsk (1980).
2. V. P. Baluda, I. I. Deyanov, M. V. Baluda, *et al., Prevention of Thrombosis* [in Russian], Saratov (1992), pp. 90-97.
3. V. V. Kupriyanov, V. A. Mironov, A. A. Mironov, *et al., Angiogenesis. Formation, Growth, and Development of Blood Vessels* [in Russian], Moscow (1993), pp. 100-104.
4. V. S. Ronin, *Lab. Delo,* No. 1, 61-62 (1983).
5. V. G. Pinchuk and K. P. Balitskii, *Eksperimental'naya Onkologiya,* **13,** 14-18 (1991).
6. P. A. Graven and F. R. Derubertis, *Carcinogenesis,* **13**, 541-546 (1992).
7. Th. J. Doghert, *Adv. Exp. Med.,* **193**, 313-323 (1985).
8. M. Hejna, M. Raderer, and Ch. C. Zielinski, *J. Natl. Cancer Inst.,* **8**, 22-36 (1999).
9. N. Jahrandi and J. S. Grecnberger, *J. Neurooncol.,* **23**, 99-108 (1995).
10. D. E. McIntyre, J. D. Pearson, J. L. Gordon, *Nature,* **271,** 549-553 (1978).