Combined Exposure to Electrochemical Lysis and Photodynamic Therapy

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A combination of electrochemical lysis and photodynamic therapy were used to attain complete resorption of M-1 sarcoma in rats; both treatment modalities were used with minimum parameters. Fotolon served as the photosensitizer for photodynamic therapy. Accumulation of the sensitizer in the tumor and normal tissue was evaluated before photodynamic therapy. Complete resorption of sarcoma in 100% cases (*vs.* photodynamic monotherapy) was attained only by the following treatment protocol: fotolon injection 50 min before electrochemical lysis (10 min) followed by photodynamic therapy. No tumor tissue was detected in morphological sections.

Key Words: *electrochemical lysis; photodynamic therapy; M-1 sarcoma*

Despite progress in modern oncology, the problem of tumor treatment is far from being solved. The therapy is effective in the majority of patients with only initial stages of cancer. The potentialities of modern oncology were essentially extended with the development of photodynamic therapy (PDT), a modern organ-sparing therapeutic method, based on selective laser exposure of tumor tissues and cells pre-sensitized by a tumorotropic stain [4,5]. However, despite the progress in development of this method, relapses sometimes emerge after therapy [6], and hence, combination of PDT with other methods used for cancer treatment seems to be a promising trend. One of these methods, though little studied by the present time, is electrochemical lysis (ECL). ECL is based on direct exposure of the tumor to permanent current until development of aseptic necrosis and delayed chemical exposure of the tumor to electrolysis products (alkali and acid) [1,7,8,10]. We found only one hospital practicing a combination of these methods (Ocular Microsurgery Research and Technological Complex named after S. N. Fedorov). This means that the method is in need of active development and introduction into clinical practice.

We studied the possibility of combined use of ECL and PDT for attaining complete resorption of M-1 sarcoma in rats with the minimum parameters of both methods.

MATERIALS AND METHODS

The study was carried out on 174 outbred rats (175-200 g) on the model of M-1 sarcoma. The tumor was transplanted subcutaneously in the hip. The experiment was started on days 10-11 (after detection of the tumor node by palpation) [3]. The hairs in the tumor growth zone were removed with depilation cream. All animals were narcotized before procedure by 0.25% sodium thiopental (0.2 ml/100 g).

Fotolon was selected as the photosensitizer (PS) for PDT. It was injected intraperitoneally in a single dose of 2.5 mg/kg or into the tumor ($^1/_2$ of its volume) [2,5]. Photodynamic therapy was carried out on an Atkus-2 therapeutic laser (P_S=0.54 W/cm^2, λ=662 nm, E=300 J/cm^2). Photodose was delivered

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perpendicularly to tumor surface via quartz monofiber rodding lightquides. The animals were kept in darkness during 24 h after PDT. Electrochemical lysis was carried out on an ECU device (Soring). Electrodes were inserted into the tumor horizontally under the tumor base at a distance of 10 mm from each other. The accumulated charge between the electrodes in tissue was 12 C. The ECL parameters were selected before: 20 mA current, 10 min duration of exposure.

Accumulation of PS in tumor and normal tissue was measured before PDT session on a Lesa-6 spectrometric complex (Biospec). Pickups were applied to the skin above the tumor in 4 points perpendicularly to the object. Accumulation in normal tissues was evaluated in the contralateral hip. Before measurements the fur was removed by depilation. The exposure duration was 1-2 sec. The tumor/normal tissue contrast index was calculated as the proportion of PS accumulation in the tumor to normal tissue.

Tumor diameters were measured before PS injection (initial values) and on days 3, 7, 10, 14, and 21 after exposure. Tumor volume has been calculated by the formula:

$$V={}^{1}/_{6}\pi\times d_1\times d_2\times d_3,$$

where: $d_{1,2,3}$ are three mutually perpendicular diameters of the tumor, ${}^{1}/_{6}\pi=0.52$ is a constant, and V is tumor volume (cm^3).

The time course of tumor growth was evaluated by the tumor absolute increment coefficient (K):

$$K=\frac{V_1—V_0}{V_0},$$

where V_0 is the initial tumor volume and V_1 is tumor volume for a certain period of observation.

The results were processed by methods of variation statistics, the significance of differences was evaluated using Mann—Whitney test. The results of experiments were compared with respective controls. The results were verified by examinations of morphological sections.

RESULTS

Study of the dynamics of fotolon accumulation in the tumor and normal tissue showed that the accumulation depended on the interval between and order of ECL and PS injection (Table 1). Injection of fotolon 20-30 min before ECL exposure led to an appreciable accumulation of PS in tissues. On the other hand, injection of PS after ECL did not lead to sufficient accumulation of the agent in tissues, irrespective of the interval between ECL and PS injection. The maximum accumulation of PS in tumor tissue in comparison with normal tissue was observed 1 h after agent injection. Based on these data, the animals were divided into 5 groups: 1) intact controls; 2) fotolon injected 1 h before PDT; 3) fotolon injection 50 min before ECL (10 min duration); 4) ECL followed immediately by fotolon injection and after 10 min by PDT; 5) ECL 24 h before PDT with preliminary (1 h before PDT) fotolon injection.

TABLE 1. Time Course of Fotolon Accumulation in Tumor and Normal Tissue

Means	Time (min) after fotolon injection						
	before injection	15	30	45	60	75	90
ECL for 10 min and fotolon after 30-60 min							
Tumor	52	45	38	41	43	41	39
Normal tissue	53	50	40	35	36	34	40
Contrast index	1.0	0.9	0.9	1.2	1.2	1.2	1.0
Fotolon (2.5 mg/kg) 40-50 min before ECL for 10 min							
Tumor	35	47	61	73	116	113	108
Normal tissue	36	44	46	56	67	65	76
Contrast index	1.0	1.1	1.3	1.3	1.7	1.7	1.4
ECL for 10 min 24 h before fotolon (2.5 mg/kg)							
Tumor	44	57	71	76	75	84	39
Normal tissue	33	36	51	49	46	52	23
Contrast index	1.3	1.6	1.4	1.6	1.6	1.6	1.6

TABLE 2. Dynamics of M-1 Sarcoma Growth after Combined ECL and PDT

Group; number of animals		Coefficient of tumor absolute increment				
		on day 3	on day 7	on day 10	on day 14	on day 21
Group 1 (control)	50	3.31±0.30	12.26±1.24	28.10±3.30	52.05±5.88	107.22±14.55
Group 2	17	0.75±0.23	5.11±1.32	8.56±2.38	16.88±4.54	21.15±9.92
		p=0.001	p=0.03	p=0.01	p=0.002	p=0.002
Complete regression of tumor, %		—	—	—	—	54
Group 3	35	-1.0	-1.0	-1.0	-1.0	-1.0
Complete regression of tumor, %		—	—	—	—	100
Group 4	18	0.11±0.11	2.20±0.75	4.61±0.98	9.11±2.41	17.21±5.25
		p=0.02	p=0.002	p=0.002	p=0.002	p<0.001
		p_1=0.2	p_1=0.05	p_1=0.15	p_1=0.16	p_1=0.63
Complete regression of tumor, %		—	—	—	—	—
Group 5	15	-1.0	1.86	3.58	3.98±2.67	11.88±3.87
					p<0.001	p<0.001
					p_1=0.08	p_1=0.63
Complete regression of tumor, %		—	—	—	—	54

Note. p: significant differences from the control; p_1: significant difference from PDT.

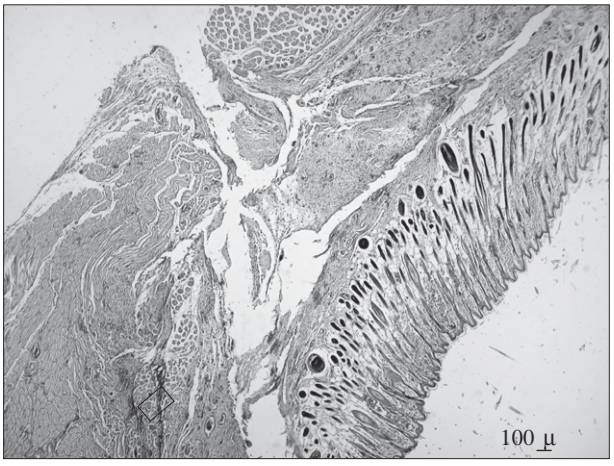

Fig. 1. A site of the rat hip on day 21 after treatment. Loose connective tissue is replaced by fibrous tissue. The vessels are dilated and plethoric (arrow). No tumor tissue detected.

A good inhibitory effect on tumor growth was observed after treatment with all combinations of ECL and PDT. The treatment protocols used in the study significantly reduced the increment of M-1 sarcoma growth during all periods of observation in comparison with the control (Table 2). However, significant difference in comparison with PDT alone were observed only in group 3. The treatment protocol used in this group led to complete resorption of sarcoma in 100% cases. No tumor tissue was detected in morphological sections (Fig. 1). Other treatment protocols did not lead to results differing from those of PDT alone either by the increment coefficient or by complete regression percentage.

Hence, combination of ECL and PDT leads to complete resorption of the tumor node with the minimum parameters of both methods. The optimal protocol of combined treatment is as follows: PS injection 50 min before ECL (10 min) and subsequent PDT.

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