Laser Heating of Sulphuretted Carbon Nanoparticles Inhibits Tumor Growth

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ABSTRACT

Antitumoral effect of a pulsed laser irradiation after intravenously injection of nanoparticles of sulphuretted carbon against colon carcinoma C-26 and sarcoma S-37 was studied *in vivo*. Damage of tumor blood vessels as result of "microexplosions" of nanoparticles is one of possible mechanisms of this effect.

***Keywords*:** nanoparticles, pulsed irradiation, tumor treatment.

1. INTRODUCTION

Effects of powerful pulsed laser radiation on light-absorbing nanoparticles incorporated in a condensed environment were studied earlier in connection with an important problem of laser damage of transparent materials [1-6]. Light-absorbing nanoparticles incorporated in a tissue can be heated by short-pulsed laser irradiation on several thousand degrees. Average tissue temperature may be kept normal if the concentration of these particles is low. Cytotoxical effects of such "microexplosions" of nanoparticles against tumor cells have been studied in vitro [7-13] and antitumoral effects against animal tumors – in vivo [14,15]. Similar method was proposed for improvement of drug delivery in tumors [16,17].
Earlier we used mice colon adenocarcinoma, Lewis lung carcinoma and ascite leukemia P-388 as tumor models and carbon black - as light-absorbing nanoparticles [14,15].
In this study other tumors and nanoparticles were tested.

2. MATERIALS AND METHODS

2.1 Preparation

Sulphuretted carbon nanoparticles (SCN) were used because they form more stable aqueous suspension than carbon black nanoparticles without surfactants. SCN were synthesized using exposition of carbon black (mark of K-354, Russia) in oleum at room temperature for one week, with subsequent dilution in water, removal of acid by repeated centrifugation, neutralization with 0.1N NaOH solution and treatment with ultrasonics. Average size of SCN in aqueous suspension several days after preparation was of about 300 nm.

2.2 Animals and tumors

C-26 colon carcinoma (female Balb/c mice) and S-37 sarcoma (male hybrid F1 mice) were used as experimental tumor models.
C-26 tumor strain was cultivated *in vitro*. Tumor cells suspension (concentration of 2 million/ml) was injected subcutaneously into an outside surface of a mouse's right thigh in an amount of 100 thousand cells.
S-37 tumor strain was cultivated *in vivo* as ascites in male SHK mice. Ascitic extract in physiological solution (concentration of 20 million/ml) was injected subcutaneously into outside surface of mouse's right thigh in amount of 1 million cells.
Aqueous SCN suspension was injected intravenously in a dose of 30 mg/kg on the 10^{th} (C-26) or 6^{th} (S-37) day after tumor transplantation when the tumor volume was equal to 75±10 mm^3.

2.3 Irradiation

Tumors were irradiated by the Q-switched Nd:YAG laser (1.06 μm wavelength, 10 ns pulse duration, 3 J/cm^2 pulse energy density, 60 or 120 pulses) right after SCN injection. Hair on the site of irradiation was removed several days before irradiation.

2.4 Processing of results

Antitumoral effects were estimated using kinetics of a tumor growth inhibition (TGI):

$$TGI = [(V_c - V)/ V_c]x100\% \quad (1)$$

where V_c and V are average tumor volume in control group (no SCN injection, no irradiation) and treated group respectively. Biologically significant tumor growth inhibition is considered to be TGI > 50%. Statistical processing of the obtained results was by methods of variation and alternative statistics and also by Student-Fisher method using "STATISTIC" program at $p \leq 0.05$.
Animal death within 7 days after treatment was the criteria of toxicity of this method.

3. RESULTS

Tumor growth kinetics in treated animals was compared with control groups (Tables 1, 2).
Biologically significant tumor growth inhibition was obtained in the groups of treated animals. C-26 tumor is more responsive to treatment. In case of S-37 tumor a magnification of the pulses number from 60 up to 120 did not improve the antitumoral efficacy.
Death of treated mice was not observed within 2 weeks after treatment.

№ gr.	SCN dose, mg/kg	Irradiation parameters		TGI on the day after treatment, %				
		Pulse energy density, J/cm^2	Number of pulses	3	5	7	11	14
1	30	3	60	76	73	58	70	48
2	30	0	0	32	28	44	24	4,7
3	0	3	60	10	16	15	-8	-19

Table 1: Antitumor effect against C-26 tumor of mice

№ gr.	SCN dose, mg/kg	Irradiation parameters		TGI on the day after treatment, %				
		Pulse energy density, J/cm^2	Number of pulses	2	5	8	12	16
1	30	3	60	72	67	57	19	-2
2	30	3	120	74	58	48	17	15
3	0	3	120	5	-2	2	-6	-13

Table 2: Antitumor effect against S-37 tumor of mice

4. DISCUSSION

Characteristic cooling time, τ, for spherical particle of radius r is approximately

$$\tau = r^2/(4\alpha) \qquad (2)$$

where α - heat diffusivity for the environment. In our case $r \cong 150$ nm, $\alpha \cong 1.5 \times 10^{-3}$ cm^2 s^{-1}. We obtain from equation (2) $\tau \cong 38$ ns. This time is more than pulse duration (10 ns) therefore heating of particle occurs near to adiabatic heating. In this case rough estimation of the particle temperature magnification, ΔT, is

$$\Delta T \cong kE/(\rho C) \qquad (3)$$

where- E - fluence per pulse, k ,ρ and C are absorption coefficient, density and specific heat capacity of particle material respectively. By substituting in equation (3) the values $k \cong 10^4$ cm^{-1}, $E = 3$ J cm^{-2}, $\rho = 1.9$ g cm^{-3}, $C \cong 0.6$ J g^{-1} K^{-1} we can obtain heating of particle near tumor surface $\Delta T \cong 26$ kK. Certainly the temperature of nanoparticle does not reach this value as additional heat wastes (transpiration of a material, formation of bubble, shockwave and radiation)

start earlier. All these effects can kill neighboring cells. Besides, new chemical products can form at high temperature. Some of them possess cytotoxicity [13]. The lower E value, the more correctly equation (3). During laser pulse a faint white radiation is observed in tumor surface layer. In such depth of tumor where E value is several ten times lower than on surface the ΔT value is several hundred K. Such heating during very short time (~10 ns) is still sufficient for formation of shock waves and microbubbles that can damage biostructures bordering upon nanoparticles. Tumors were irradiated short time after injection when SCN are in blood mainly. In this case the targets of effect are blood vessels walls probably. Really, after irradiation session the tumour metachromatism caused likely by microhemorrhages as a result of capillars damage is observed.

Increase of a period between SCN injection and an irradiation results in decrease of treatment efficacy. This is likely due to short time of circulation in blood for such rather large particles (300 nm) that are deleted rapidly from blood by reticuloendothelial system. Very small nanoparticles can circulate during long time in blood but very high irradiation power is necessary for their heating. For example let's consider SCN of radius r = 15 nm. From equation (2) we obtain τ = 0.4 ns, that is, it is much less than pulse duration. In this case heating of nanoparticle is quasistationary:

$$\Delta T = \sigma P/(4\pi ar) = \sigma E/(4\pi ar\Delta t) \qquad (4)$$

where σ - cross-section of light absorption for particle, P – fluence rate, a -thermal conductivity for the environment, Δt - pulse duration. In our case $\sigma \cong 5\text{x}10^{-13}$ cm^2, $a = 6\text{x}10^{-3}$ W cm^{-1} K^{-1}, Δt = 10 ns. From equation (4) at E = 3 J cm^{-2} we obtain maximum ΔT value for such nanoparticle near tumor surface: ΔT = 1.3 kK. In tumor depth ΔT = 130 K if E = 0.3 J cm^{-2}. Generally E value can be increased several times therefore the tests with smaller nanoparticles are advisable. So it is possible to increase period between injection and an irradiation. In this case nanoparticles can be aimed to tumor cells from blood vessels and new effects can occur.

Other way for increase of time of nanoparticles being in blood is their conclusion in a shell of a suitable material (f. e. polyethylene glycol).

Besides it is advisable to select materials for nanoparticles with k and σ values that are higher.

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