Study of Tumour and Surrounding Tissue Heating with Near-Infrared Radiation after the Injection of Gold Nanoparticles into the Tissue

Vadim D. Genin[1*], Elina A. Genina[1,2], Alla B. Bucharskaya[3], Marina L. Chekhonatskaya[3], Georgy Terentyuk[3], Daria K. Tuchina[1], Nikolay G. Khlebtsov[4], Valery V. Tuchin[1,2,5], and Alexey N. Bashkatov[1,2]

[1] Saratov State University, 83 Astrakhanskaya Str., Saratov 410012, Russia
[2] Tomsk State University, 36 Prosp. Lenina, Tomsk 634050, Russia
[3] Saratov State Medical University named after V.I. Razumovsky, 112 Bol'shaya Kazachya Str., Saratov 410012, Russia
[4] Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences, 13 Prosp. Entuziastov, Saratov 410049, Russia
[5] Institute of Precision Mechanics and Control, Russian Academy of Sciences, 24 Rabochaya Str., Saratov 410028, Russia

*** e-mail:** versetty2005@yandex.ru

Abstract. We study the heating kinetics in transplanted model tumours after the intravenous injection of suspension of gold nanorods having the concentration from 400 to 1200 μg/mL under the laser irradiation at the wavelength 808 nm during 15 min. The object of study were 40 outbred white laboratory rats with transplanted liver cancer tumours (Cholangiocarcinoma PC1). The obtained results allow the optimisation of the gold nanorods injection technique at different degrees of vascularisation of tumour tissues aimed to provide the maximal heating of tumours by the laser radiation. It is shown that the maximal increase of the tumour temperature is related to the maximal accumulation of gold nanorods in the tumour tissues, observed in the case of its high vascularisation.

Keywords: tumour; NIR laser irradiation; photothermal therapy; gold nanorods.

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1 Introduction

The growth of oncological diseases stimulates extensive development of both early tumour diagnostics and therapy methods. Earlier it has been shown that the heating of a tumour leads to its death and the exposure regimes for tumour damage have been determined, namely, 120 minutes at 42°C, 60 minutes at 43°C, 30 minutes at 44°C and 15 minutes at 45°C [1]. However, the hyperthermia is restricted by low selectivity, which leads to significant damage of healthy tissues adjacent to the tumour. The laser hyperthermia is a promising method of fighting tumours that provides more heating locality than the traditional hyperthermia, which allows damage reduction in surrounding healthy tissues [2].

The use of sensitizers allows even higher selectivity of laser hyperthermia due to the reduction of the laser radiation power to the level safe for the healthy tissues adjacent to the tumour [3-7]. Nanoparticles possessing plasmon resonance near 800 nm are effectively applied as sensitizers, which makes it possible to use lasers with the appropriate generation wavelength as sources of radiation [8, 9]. It has been shown that gold nanorods (GNRs) are promising agents for photothermal tumour therapy (PTTT) due to their long-term circulation in blood flow [10], colloidal stability, easy spectral tuning of plasmon resonance [6], and efficient light-to-heat energy conversion [11].

In spite of multiple studies in the field, this kind of therapy requires optimising the dose of nanoparticles and their injection method, as well as the technique of heating them with near-infrared optical radiation, i.e., the power, exposure time, and depth of penetration of laser radiation into the tissue. The authors of Ref. [12] proposed the optical monitoring of accumulation of nanoparticles in the tumour using the optical coherence tomography. However, this method is applicable only to surface tumours, since due to the strong scattering of light in tissues the optical probing depth is restricted to 0.3-1.5 mm, depending on the illumination source wavelength [12-15]. Among other factors that affect the efficiency of using GNR for PTTT an important role is played by the degree of tumour vascularisation, i.e., the development of its blood vessel system, since the degree of vascularisation directly affects the accumulation of nanoparticles in the very object of study, the tumour tissue [16-18]. In turn, the accumulation of nanoparticles in the tumour tissue will determine the tumour temperature in the course of laser heating, directly affecting the PTTT efficiency [8]. Since to estimate the vascularisation of the tumour one needs the probing depth of about 10 mm, this problem can be solved using the Doppler USI. Unfortunately, in spite of its importance, the problem is still far from its final solution. Hence, the aim of the present paper is to study the heating kinetics in tumours with different vascularisation after the intravenous injection of gold nanorods.

2 Materials and methods

The object of study were 40 laboratory outbred male rats with the body mass 200 ± 20 g. The animals were kept in the Shared Cervices Centre of Saratov State Medical University named after V.I. Razumovsky under the standard vivarium conditions with fixed illumination regime. The animals were treated in accordance with the rules of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and the International Guiding Principles for Biomedical Research Involving Animals [19].

The experimental model of rat cholangiocarcinoma PC1 was obtained by subcutaneous injection of tumour cell suspension (2×10^6 cells/0.5 mL of Hanks solution). When the tumour volume achieved nearly 3 cm^3, the rat tumours were examined using the US system Voluson E8 Expert (GE Healthcare, USA) in the Doppler mode at the frequency 7.2 MHz. The 3D Doppler US imaging allowed the assessment of tumour vascularisation degree using the standard technique [20]. The estimation of the tumour vascularisation degree was based on the analysis of the resistance index (RI) of the tumour blood vessels, defined as the difference between the peak systolic velocity (PSV) and the end diastolic velocity (EDV) divided by the peak systolic velocity, RI = (PSV - EDV) / PSV. The values of PSV and EDV were determined in the course of dopplerography. It was found that at a certain stage of growth (RI ≤ 0.3) the developed afferent vessels with increased flow velocity appear in the tumour [21, 22].

Basing of the degree of tumour development, the animals were divided into two parties: 1) with poorly developed tumour vascularisation (24 animals with RI > 0.3); 2) with well-developed tumour vascular system (16 animals with RI ≤ 0.3).

Within these parties, the animals were randomly divided into 6 and 4 groups, respectively (10 groups overall, 4 rats in each group). Before the irradiation, the rats of eight groups were subjected to intravenous injection of GNR suspension. The experimental conditions are described in Table 1.

The gold nanorods were synthesised and functionalised with thiolated polyethylene glycol (molecular weight 5000, Nektar, USA) using the methods described in Refs. [11, 23]. The GNR geometric parameters determined by means of transmission electron microscopy (Libra-120, Carl Zeiss, Germany) amounted to 41 ± 8 nm (length) and 10 ± 2 nm (diameter). For the study, we used the GNR suspension with the concentration 400 μg/mL, which corresponds to the optical density 20 at the wavelength 810 nm. To increase the heating temperature, the animals of Groups 5 and 6 received the suspension with double GNR concentration, i.e., 800 μg/mL, 48 and 24 hours before the irradiation, respectively.

Table 1 Description of groups of animals used in the experiments (4 rats in each group).

Group number	Regime of injecting the GNR suspension doses	Total suspension dose
	Poorly developed tumour vascular system	
1	Irradiation without injecting nanoparticles	-
2	Single injection (1 mL) 24 hours before irradiation	400 μg/mL
3	Double injection (1 mL each, 400 μg/mL) 48 and 24 hours before irradiation	800 μg/mL
4	Triple injection (1 mL each, 400 μg/mL) 72, 48, and 24 hours before irradiation	1200 μg/mL
5	Single injection (1 mL) 48 hours before irradiation	800 μg/mL
6	Single injection (1 mL) 24 hours before irradiation	800 μg/mL
	Well-developed tumour vascular system	
7	Irradiation without injecting nanoparticles	-
8	Single injection (1 mL) 24 hours before irradiation	400 μg/mL
9	Double injection (1 mL each, 400 μg/mL) 48 and 24 hours before irradiation	800 μg/mL
10	Triple injection (1 mL each, 400 μg/mL) 72, 48, and 24 hours before irradiation	1200 μg/mL

To irradiate the tumours we used the diode laser LS-2-N-808-10000 with the wavelength 808 nm (Laser Systems Ltd., Russia). The exposure time was 15 min with the power density 2.3 W/cm^2. The laser spot area at the skin surface was ~0.5 cm^2. The temperature control of the tumour heating was implemented using the IR visualizer IRI4010 (IRYSYS, UK) with the interval of 30 s. Before all procedures the rats were anesthetised with Zoletil 50 (Virbac, France) dosed 0.05 mg/kg.

3 Results and Discussion

The heating kinetics of the experimental tumours and adjacent tissues is presented in Fig. 1. As seen from the figure, the laser irradiation of tumours without preliminary injection of GNR in Groups 1 and 7 caused insignificant increase of the tumour surface temperature approximately to 40°C, independent of the tumour vascularisation degree. In the case of poorly developed vascular blood flow, a single injection of the GNR suspension also caused the temperature growth not exceeding 40°C under the laser irradiation (Group 2). To our opinion, this is because no accumulation of a sufficient amount of nanoparticles occurs in the tumours due to the low vascularisation of the tumour tissue. This assumption is confirmed by the fact that the mean temperature of skin surface covering the tumour in Group 8 (with well-developed vascular blood flow) at the same dose as in Group 2 (with poorly developed vascular blood flow) achieved the value of 49.9±4.3°C, which is explained by considerable accumulation of particles in the tumour tissue.

Besides that, it is clearly seen that the dose doubling and tripling (Groups 3-6) has practically no effect on the temperature growth. The maximal temperature approached the values from 47°C to 52.2°C upon average, which allows a conclusion that in these groups no sufficient accumulation of nanoparticles in tumours occurred, because of the low tumour tissue vascularisation.

Note that in Group 9 we observed the temperature increase to 59.4 ± 10.4°C at the same injected GNR dose, as in Groups 3, 5, and 6. This group differed from Groups 3, 5, and 6 by higher vascularisation of tumour tissues, due to which in Group 9 the accumulation of nanoparticles appeared sufficient to provide significant tumour heating.

Group 10 has demonstrated the average temperature growth in the region of the tumour to 68.2°C, which, to our opinion, is related to the maximal GNR accumulation due to both the high total dose of the injected suspension and the high vascularisation of the tumour tissues. This assumption is confirmed by the data of Ref. [24], where it was shown that the maximal accumulation of nanorods in the tumour is observed in the case of triple injection of nanoparticles.

In Ref. [25] it was shown that the direct intratumoural injection of GNR suspension with the concentration 400 μg/mL followed by the irradiation of tumours with laser light at the wavelength 808 nm led to the increase of temperature in the heating zone to 65.5 ± 5°C. With this method of injection, the maximal amount of nanoparticles was accumulated in tumour. Since in our experiments (Groups 2-6, 8-10) the injection of GNR suspension was intravenous, the maximal concentration could be achieved only in the case of well-developed vascular system of the tumour.

To approximate the heating kinetics, presented in Fig. 1, we used the empirical equation:

$$T(t) = A_1\left(1-\exp\left(-\frac{t}{\tau_1}\right)\right) + A_2\left(1-\exp\left(-\frac{t}{\tau_2}\right)\right) + T_0, \quad (1)$$

where T_0 is the initial temperature (before heating) having the mean value of 32.9 ± 1.7°C, A_1 and A_2 are empirical constants, τ_1 and τ_2 are the constants that

characterise the heating rate for skin and tumour, respectively. The first term describes the heating kinetics of the adjacent tissues, and the second term describes the heating kinetics of the tumour itself.

The approximation parameters are presented in Table 2.

The analysis of the approximation parameters shows that after the intratumoural injection of GNR suspension the characteristic time of tumour heating is minimal. In this case, the degree of tumour vascularisation does not matter, since the nanoparticles are injected into the interstitial fluid. On the contrary, the characteristic time of heating for the surrounding tissues is maximal, which can be because the particles are localised in the injection sites, i.e., are not distributed uniformly over the tumour volume. Thus, sufficiently strong and fast heating of the localisation sites of nanoparticles occurs in combination with rather slow heat penetration into the surrounding tissues, which is in good correlation with the results of Ref. [26].

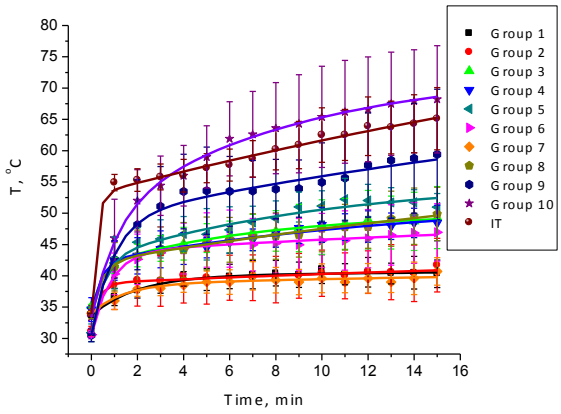


Fig. 1 Heating kinetics of experimental tumours and adjacent tissues stimulated by near-infrared radiation (the IT group comprises the rats subjected to a single intratumoural injection of GNR suspension with the concentration 400 μg/mL [25]).

In the first and the seventh groups of animals (irradiation without injection of nanoparticles), the value of τ_2 is relatively large. In the case of poorly developed vascular system (Group 1), the time necessary to heat the tumour is smaller than in the case of well-developed vascular system (Group 7), since the heat transfer from the heated region with the blood flow is weaker in Group 1. The value of τ_1, on the contrary, is smaller in Group 7, which confirms the greater velocity of heating the tissues surrounding the tumour at the expense of heat transfer from the heated region to the surrounding tissue with the blood flow.

The large deviation of the approximation parameters from the mean value, to our opinion, is related to the variety of structural features of tumours in individual animals and the location of blood vessels with respect to the irradiation zone. The latter is of primary importance in the case of intravenous injection of GNR. To determine these parameters with greater precision, one has to increase the sample volume, i.e., the number of studied animals, which is planned by the authors in future. However, the presented results of pilot experiments allow the observation that, overall, the increased vascularisation reduces the rate of tumour heating due to the increased temperature gradient from the irradiation zone.

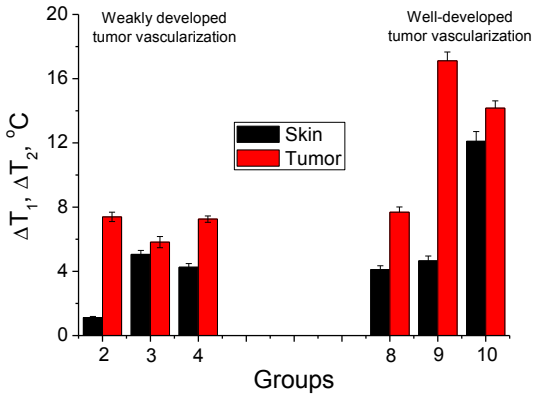
The data of Table 2 allow the estimation of the partial contributions ΔT_1 and ΔT_2 to the experimentally recorded increment of temperature from the tumour itself and from the surrounding tissues, respectively, during the irradiation time ($0 < t < 15$ min). Here

$$\Delta T_1 = \frac{A_1\left(1-\exp\left(-\frac{t}{\tau_1}\right)\right)}{N},\ \Delta T_2 = \frac{A_2\left(1-\exp\left(-\frac{t}{\tau_2}\right)\right)}{N},$$

and $N = 31$ is the number of time points at which the temperature was measured. In Fig. 2a, the groups with equal volume of injected GNR suspension differing by the tumour vascularisation degree are presented in pairs.

Table 2 Parameters for approximating the experimental data.

Group number	Approximation parameters				
	T_0, °C	A_1	A_2	τ_1, min	τ_2, min
1	33.7±0.3	3.0±0.2	4.6±0.9	30.3±12.6	1.4±0.96
2	31.1±0.8	5.7±0.9	7.7±3.1	32.7±6.5	0.3±0.003
3	35.2±0.2	8.7±2.5	6.2±4.1	6.9±1.1	0.5±0.35
4	34.6±1.9	10.3±3.6	7.6±1.6	14.3±12.0	0.4±0.03
5	30.9±1.4	12.9±2.8	11.6±0.9	10.2±4.4	0.5±0.03
6	30.6±0.4	7.1±2.1	13.1±3.4	28.9±2.2	0.8±0.18
7	34.0±0.3	1.4±0.4	6.3±1.9	28.8±0.5	1.9±0.06
8	33.9±0.8	19.4±4.9	8.1±3.5	30.5±11.1	0.5±0.03
9	30.5±1.1	25.6±17.8	18.6±4.7	32.3±18.8	0.96±0.05
10	33.4±1.9	23.8±8.0	15.7±4.1	8.7±3.4	1.1±0.8
IT	33.9±0.2	40.9±13.0	19.0±0.9	41.9±6.9	0.2±0.01



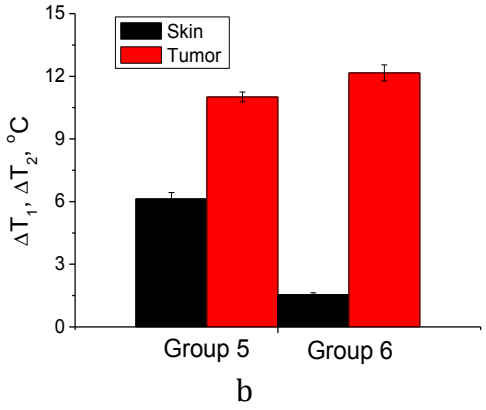


Fig. 2 Partial contributions ΔT_1 and ΔT_2 to the experimentally recorded temperature increment from the tumour and the surrounding tissues, respectively, in the groups of animals with equal volume of injected GNR suspension.

In Fig. 2a it is clearly seen that in the case of weakly expressed vascularisation degree the tumour heating due to GNR does not provide the temperature of the order of 45°C [1], necessary for the damage of cancerous cells (Groups 2-4), taking into account that the mean recorded initial temperature for all groups being 32.9 ± 1.7°C (see Table 2). Similar situation is observed for the animals with developed vascularisation of tumour after a single injection (Group 8). With double and triple increase of the GNR doze (Groups 9 and 10), the temperature necessary for damaging the cancer cells is achieved. However, for Group 10 we see unacceptable temperature growth in the tissues surrounding the tumour, which, to our opinion, is related to the escape of GNRs from the tumour vessels and their distribution in the surrounding tissues. This conclusion is confirmed by the data presented in Fig. 2b. It is clearly seen that when the GNRs are injected 2 days before the irradiation, the observed heating of surrounding tissues differs from the situation, when the same doze was injected only 1 day before irradiation. In the latter case, the growth of temperature in the surrounding tissues is essentially lower.

4 Conclusion

The obtained results allow a comparison of the techniques of gold nanorods injection providing the maximal heating of tumour with laser radiation. The maximal tumour temperature increase is related to the maximal accumulation of gold nanorods in the tumour. It is observed after triple intravenous injection of 400 μg/mL (the total dose 1200 μg/mL) 72, 48, and 24 hours before irradiation, provided that the vascular system of the tumour is sufficiently developed, since the accumulation of nanoparticles in the tumour tissue depends on its vascularisation degree. It is shown that in tumours with well-developed vascular system a trend that can be detected is the growth of the partial contribution of tumour heating under the increase of the GNR dose. However, when using a triple GNR injection, one can observe a damage of the surrounding tissues. Thus, to our opinion, the double injection of 400 μg/mL (the total dose 800 μg/mL) 48 and 24 hours before the irradiation is more preferable, if the vascular system of the tumour is sufficiently developed.

One of the ways to evaluate the tumour vascularisation degree is to use the Doppler USI. For the values of the tumour vessels resistance index ≤ 0.3 the vascularisation is sufficient for the PTTT procedure. Thus, the Doppler US visualisation of blood flow can be used to optimise the time of GNR injections before the PTTT with the purpose of improving the procedure efficiency.

Disclosures

All authors declare that there is no conflict of interests in this paper.

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