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Original research paper

THE PHOTON CAPTURE THERAPY MODEL FOR *IN VIVO* AND *IN VITRO* STUDIES USING Au NANOCOMPOSITES WITH THE HYALURONIC ACID BASED COMPOUNDS*

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Abstract. *This study provides the quantitative assessment of the photon-capture therapy efficiency using the X-ray facility and Au nanocomposite in solutions based on hyaluronic acid and melanin. The RBE using 10 % survival criteria for murine B-16 melanoma cell cultures was 1.5. The rats with implanted sarcoma M-1 were locally irradiated with 28-32 Gy of tumor dose. Irradiation in 28 Gy of cell cultures with the 4 mg of Au-based solution administered 15 minutes before irradiation, showed the similar efficiency as conventional 32 Gy. The skin reaction yield is dependent only on the absorbed dose. The therapeutic gain using the tumor growth suppression factor was 1.35, which is comparable to the RBE of B-16 cell cultures.*

Key words: *Antitumor efficiency, Au distribution, gold nanoparticles, hyaluronic acid, melanoma B-16, photon capture therapy, sarcoma M-1, X-ray*

1. INTRODUCTION

One of the possible solutions to improve the radiotherapy efficiency is to increase the dose deposited into the tumor while reducing the dose in the adjacent healthy tissues. This issue can be resolved using a binary therapy method, in particular, the photon capture therapy (PCT). The metals selectively accumulated within the tumor are coupled with the X-rays' additional interaction, which finally results in the emission of the short range secondary ionization particles leading to the tumor dose enhancement [1, 2]. The wide spread of the PCT method into the clinical practice is limited mostly by the lack of effective drugs and substances satisfying the method requirements: higher concentration in the tumor and precise dose escalation that could lead to the tumor growth suppression effect. The potentially applicable compounds are the high-Z (>53) elements and polysaccharides [3], but the physiologically inert particles such as Gold NanoParticles (AuNP) can also be used due to their unique opto-electrical properties [4]. The study is aimed at the biological efficiency evaluation of the experimental PCT using Au-based compounds with hyaluronic acid and melanin.

2. MATERIALS AND METHODS

The photon capture events on Au and the dose deposit of X-ray source inside the water and tissue-equivalent phantoms has been simulated using MCNP Monte-Carlo code [9]. The superficial tumor approximation has been used. The simulated monodirectional X-ray source is the disc located near the phantom. The Intel Xeon E5506 2.13 GHz was used for the simulations. The X-ray source energy distribution corresponds to the real X-rays facility based on the bremsstrahlung photons. The 60 and 70 keV peaks correspond to the W (wolfram) anode emission spectrum. The max energy is 210 keV. The concentration of Au within the compound for PCT has been chosen by the evaluation of the minimal significant metal concentration to initiate the capture events on Au (no less the 10 mg per 1 g of tumor tissue) [10]) and our own *in vivo* experimental data of the dose enhancement with the low Au concentration (2 mg per 1 g of tissue) [5].

Au nanocomposite has been synthesized by the International scientific research centre of innovative technologies "Martinex" (Moscow, Russia). The bioactive composites were produced using the Au recovered from its salt in the viscous hyaluronic acid solution with melanin and follow-up boiling and lyophilization. The melanin content is 80± 0.2 mg per g of original substance; the Au content is 20.8 ± 0.3 mg per g of original substance. The melanin is used because of its ability to recover and stabilize the Au particles due to creating functional connections between melanin and Au in the form of chelates or unstable complexes [6].

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The *in vitro* study of the relative biological efficiency (RBE), due to dose enhancement in the Au capture events in the 2-8 Gy dose range, were performed with the clonogenic activity tests using mice melanoma B-16 cell cultures. The RBE was assessed on the 10 % survival level after the incubation in the Au nanocomposite solution.

The mice with the implanted melanoma B-16 were used for *in vivo* trials to assess the metal distribution within the tumor. The volume of the tumor at the moment of the drug administration was 1.1 ± 0.2 cm^3. The Au content in the bioassays was measured using the optical emission method with inductively coupled plasma (ICP) via atomization using ICP-OES (Varian Med. Industries, USA).

The cell culture (murine melanoma B-16) and rats with the implanted sarcoma M-1 were irradiated on the RAP 220-5 X-ray facility (anode voltage – 180 kV, average current – 5 mA). The animals were locally conventionally irradiated with the 28, 32 and 36 Gy dose X-ray and with 28 Gy using intratumoral administration of the Au nanocomposite (4 mg metal per rat 15 min before irradiation). The biological efficiency was assessed as the tumor growth suppression factor and the skin reaction yield.

3. Results

The concentration of Au within an auriferous compound which does not cause a toxic effect for B-16 cells has been evaluated in the *in vitro* study. The minimal (about 15 %) inhibited cell division after 24 hrs of incubation in the Au nanocomposite substratum with hyaluronic acid and melanin was assessed as 50 - 100 μg Au per ml. The 100 μg Au per ml concentration and 24 hr incubation time has been chosen for PCT. The X-ray irradiation with 4 Gy resulted in 75 % viability suppression of B-16 melanoma cells, while the 95 % suppression has been observed if the cells were incubated in the auriferous solution, in comparison to the control one. The dose-survival data (see Table 1) for conventional X-rays was approximated using only the linear-quadratic model (LQM: $\alpha = 0.295 \pm 0.034$, $\beta = 0.041 \pm 0.005$, $R^2 = 0.999$) while for PCT both the linear model (LM: $\alpha = 0.635 \pm 0.021$, $R^2 = 0.998$) and LQM ($\alpha = 0.514 \pm 0.015$, $\beta = 0.044 \pm 0.006$, $R^2 = 0.999$) were used.

Table 1. Survival of melanoma B-16 cells after X-ray irradiation and the introduction of Au in the nanocomposite with hyaluronic acid

Dose, Gy	Survival, %	
	X-ray	X-ray+Au
2	56.4 ± 5.1	30.0 ± 2.7
4	20.1 ± 1.8	5.9 ± 0.5
5	7.1 ± 0.6	2.7 ± 0.2
6	3.5 ± 0.3	1.4 ± 0.1
8	0.8 ± 0.1	0.42 ± 0.04

The RBE assessment is the most valuable study within the radiobiological trials and the most essential tool to reproduce the practical needs aimed at the radiotherapy schemes' development. The RBE using 10 % survival criteria for B-16 cells is 1.5, while a similar study, using the same object but with other incubation properties, was much lower – 1.05 [2]. Even a higher RBE of 2.0 has been observed on the 2 Gy exposure level of mice melanoma cells (which is the fraction standard in the conventional radiotherapy schemes).

That is, the RBE assessment using cell cultures is dependent on the test system used and mostly could not be unified under the experimental conditions. That is the reason to use animals for such studies.

The simulation of binary therapy for the Au concentration in a range from 1 to 10 mg per 1 g of tissue leads to the linear increase of the dose deposit ratio from metal concentration. Twice the absorbed dose value can be reached for PCT on an X-ray facility with 10 mg of Au per 1 g of tumor tissue [11]. The suggested explanation for this effect is the following: the X-ray particles interact with electron shells of the atoms leading to additional electrons' emission due to Auger cascades and Coster-Kronig transition. With the increase of tumor depth, the absorbed dose decreases due to lots of X-rays being absorbed within the previous adjacent tissue layers.

The accumulation curves for both the whole tumor calculation and normalized to 1 g in the tumor tissue have shown the same dynamic following the single exponential model: $t1/2 = 0.3$ h, $R^2 = 0.999$. The Au concentration in the tumor after 0.5 hrs from administration was measured as 17.5 % and it decreased with time. This data was later used as the criterion to choose the time between drug administration and irradiation [12]. Some researches show the significance of the shape and size of the AuNP on the metal accumulation inside tumor [7, 8]. The highest Au concentration in the tumor (sufficient for the effective application of PCT) was obtained for the size of AuNP of less than 10 nm in the first 5 min after administration. Later, the Au content significantly decreased. For the larger AuNP size (100 nm), a slightly lower decrease of concentration with time was observed, which indicates a higher uptake of Au within the tumor.

The tumor growth dynamic measured for experimental rats with sarcoma M-1 showed similar results for 32 Gy X-ray (conventional) and experimental PCT with 28 Gy + 4 mg of Au per tumor groups: the integral under the growth curve area (S) was assessed as 31.0 and 31.3 arbitrary units respectively (see Fig. 1, Table 2). The smaller area (S) corresponded to the higher antitumor efficacy. The conventional 28 Gy irradiation has shown a much lower antitumoral efficacy. The relative therapeutic gain of the tumor growth (measured as $Vrel = Vti/V0$ where Vti – tumor volume on i-th day after irradiation, cm^3, V0 – tumor volume before irradiation, cm^3) after 27 days was about 35 %.

The local 30 Gy irradiation for mice with EMT-6 carcinoma and single intravenous administration of nanoparticles with the size of 1.9 nm (corresponds to 1.35 mg of Au per 1 g of mice mass) has shown [8] that 90 % of animals had notable or complete tumor regression a month after irradiation while the size of the tumor for the group of mice without AuNP drug

increased 5 times. In our study, we observed an 11 times higher tumor volume for the control group (without irradiation).

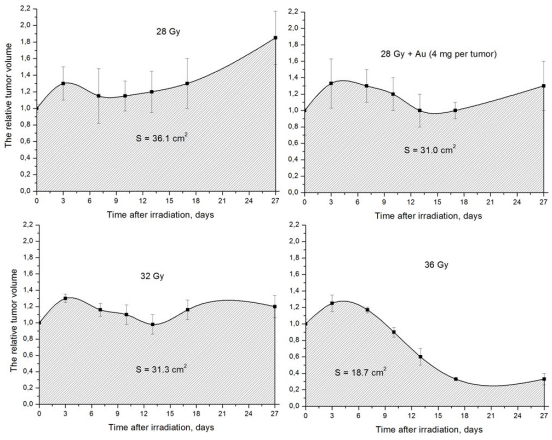

Figure 1. Dynamics of the tumor growth of sarcoma M-1 (Vrel) (depending on the X-ray dose)

The measured skin reaction yield for conventional 28 Gy X-ray irradiation does not show any notable differences in comparison to the experimental PCT group (28 Gy + 4 mg Au by intratumoral administration). At the same time, the skin reaction yield was 19 % higher for the mice irradiated with 32 Gy in comparison to 28 Gy, for 36 Gy – 50 % higher, (in this dose, the maximal antitumor efficiency was shown).

Table 2. Mean value of area (S, cm²) under the curve of the changes Vrel and ratio to S28Gy, 10, 17 and 27 days after the irradiation of rats with sarcoma M-1

Dose, Gy	S (cm²) on day after irradiation			Ratio (S/S28 - 1)100 on day after irradiation, %		
	S10	S17	S27	10	17	27
28	11.8	20.3	36.1	0	0	0
28 + 4 mg Au	11.8	19.2	31.0	0	-5.4	-14.1
32	12.5	19.8	31.3	+5.9	-2.5	-13.3
36	11.3	15.4	18.7	-4.2	-24.1	-48.2

4. Conclusion

The assessed contribution of the photon capture events to the total absorbed dose using an X-ray source was comparable for *in vitro* and *in vivo* cases. The practical therapeutic efficiency of the PCT using Au nanocompounds within the hyaluronic acid and melanin solutions in our study was 3 times higher than the supposed physical dose enhancement simulated with the Monte-Carlo approach. This once again underlines the key importance of the radiobiological studies in the radiotherapy technology improvement.

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