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# The study of hyaluronic acid compounds for neutron capture and photon activation therapies

**Research Article**

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**Abstract:** The therapy of radioresistant tumors remains an urgent problem in medicine. To solve this problem neutron capture therapy (NCT) and photon activation therapy (PAT) are used. The essential feature of this such technique is the uptake of tumor chemical elements, which interact with thermal neutrons (NCT) and X-rays (PAT). The aims of the investigation were to study a biodistribution of the complexes of hyaluronic acid with boron (3 mg B $mL^{-1}$) and gold (20 mg Au $mL^{-1}$) in mice with melanoma B-16 after intratumoral administration. An optimal time for NCT was 30 minutes after administration when boron concentration in the tumor was more than 30 $\mu g\ g^{-1}$ and exceeded boron content in surrounding tissues. The maximal gold content in tumor (180-260 mg $g^{-1}$) was obtained in 30 minutes after the preparation introduction. The highest ratios of gold in tumor and surrounding tissues (a necessary condition for forming of the local absorbed dose in tumor) was obtained in 0.5 and 1 h. On the basis of the data obtained, it is possible to assume the perspectives of the non-toxic boron compounds for use in NCT and the gold compounds for use primarily as contrast agents for diagnostic purposes.

**Keywords:** *Boron • Gold • Melanoma B-16 • Intratumoral Administration • Biodistribution • NCT • PAT*



## 1. Introduction

In the present day, the therapy of radioresistant malignant tumors remains a persisting problem in the medicine. The usage of binary methods of radiation exposure – the neutron capture therapy (NCT) and the photon activation therapy (PAT) – are two possible ways of solving this problem.

The neutron capture therapy is provided by interaction of two components: thermal neutrons produced in biological tissue due to thermalization of epithermal neutrons (neutron sources – reactors or proton accelerators with target of special materials), and the compound delivers a chemical element with high capture cross section of thermal neutrons to the tumor cells. $^{10}B$ found the practical application in NCT originates from its physical and chemical properties as typical neutron capture reaction products (α-particles and $^{7}Li$ recoil nuclei) and permit local release of energy commensurable to the diameter of the cell [1]. Application of boron compounds selectively accumulated in tumor allows us to provide the lethal effect after the capture reaction, primarily in tumor cells. Currently, two compounds are used in the clinical practice of the boron neutron capture therapy (BNCT): p-boronophenylalanine (BPA) and mercaptododecaborate sodium ($Na_2B_{12}H_{11}SH$), but these compounds are not ideal for BNCT and therefore studies of new and more effective boron compounds is conducted [2-4].

The physical principle of photon activation therapy (PAT) consists of increasing the local release of energy (photoelectrons and Auger-electrons) that result from the photoeffect arising when the soft X-ray radiation interacts

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with atoms of elements with large atomic number Z [5-7], herewith prospects for use of gold nanoparticles in the PAT are shown in several publications [8,9]. This type of radiation therapy is most suitable for wide use in oncology practice since X-ray sources are mobile, relatively cheap and do not require special rooms with a powerful biological protection when operating and that creates conditions for PAT widespread use in the treatment of cancer patients.

The main requirements for successful BNCT and Au-PAT treatment are: (a) boron- and gold-containing compound have to be delivered to the neoplastic tissue to provide specific and selective tumor targeting, and (b) the amount of boron-10 and gold atoms concentrated inside or around the cancer cells must be sufficient for the therapeutic purpose. For similar dose enhancements, Au-PAT requires local contrast agent concentrations which are about 500-1000 times more than those required for BNCT.

Thus, the distinctive feature of NCT and PAT is the need to accumulate in the tumor zone the chemical elements interacting with the thermal neutrons for NCT and X-rays for PAT with the local release of secondary radiation, which allows increase of the absorbed dose entirely in the interaction site (tumor).

To transport the drugs effectively through the cellular membrane and to deliver them into the intracellular environment, several interesting smart carrier systems based on both synthetic or natural polymers have been designed and developed [10,11]. In recent years, hyaluronic acid (HA) has emerged as a promising candidate for intracellular delivery of various therapeutic and imaging agents because of its innate ability to recognize specific cellular receptors that are over expressed on diseased cells [12,13].

The hyaluronic acid molecule is an anionic linear heteropolysaccharide constructed of regularly alternating D-glucuronic acid and *N*-acetyl-D-glucosamine residues. HA has physicochemical and biological properties that hatdetermine the biocompatibility, biodegradation without reactive toxic product formation. Also HA has attracted much attention in tumor-targeted delivery because of its ability to specifically bind to various cancer cells that overexpress CD44 receptor [14]. Therefore, the synthesis of compounds on the basis of HA is a prospective direction to search for compounds for NCT and PAT. For this, new complexes composed of a variety of chemical elements boron, gadolinium, gold, *etc.* are being developed [12,15].

The aim of this report was to study the biodistribution of new complexes of hyaluronic acid with boron and gold in organs and tissues of tumor-bearing animals to assess the feasibility of their use as agents for NCT and PAT.

# 2. Experimental Procedures

## 2.1 Compounds

Martinex Research Centre (Moscow, Russia) provided the compounds for the studies. The compound on the basis of hyaluronic acid and stable isotope $^{10}B$ (Borgyal, 3 mg B $mL^{-1}$), and also gold (20 mg Au $mL^{-1}$) containing complexes of hyaluronic acid and melanin with different concentration – P-1, P-2 preparations. The synthesis of boron compound based on hyaluronic acid was presented in previous study [16].

The synthesis of the gold compounds was performed by the following scheme: the extrusion resulting mixture of HA and melanin (weight ratio 1:1) with a total mass of 0.4 g was dissolved in 9.6 ml of distilled water. Then 4% solution of chloroauric acid in 10.0 ml was gradually added into the HA-melanin solution under boiling and vigorous stirring. There were two melanin-elemental gold solutions: one with weight ratio 1:1 (P-1) and another - 2:1 (P-2 and each solution was evaporated to 10 ml. During the synthesis, the gold is reduced to nanosized gold (0, +2, +3) interacting with hydroxyl groups of the HA salt(s) to give HA esters on one side and on the other side with the carboxyl, amino-, o-hydroquinone, o-quinone and semiquinone, indole quinone group of compounds from the group of melanins to give stable chelate polycomplexes: melanin-gold-HA; melanin-HA-gold; melanin-gold; HA-gold and mixtures thereof.

## 2.2 Animals and tumors

F1 ($CBAxC_{57}Bl_6$) (20-22 g) mice were subcutaneously injected in the right thigh with $10^6$ murine B-16 melanoma cells suspended in 0.2 ml of medium.

The boron compound Borgyal with concentration of 3 mg boron per 1 ml (0.1 ml) was intratumorally introduced to mice on the 12$^{th}$ day after grafting (tumor volume ~ 0.8-1.2 $cm^3$). Eight groups with seven mice in each were formed. Samples of tumor, blood, muscle, skin, kidneys, liver, spleen, and lungs were taken from decapitated under anesthesia mice for subsequent analysis of boron content in 0.25, 0.5, 1, 3, 6, 9, 12, and 24 h after compound administration.

The gold compounds (P-1 and P-2) with concentration of 20 mg gold per 1 ml (0.1 ml) was intratumorally introduced to mice on the 12$^{th}$ day after grafting (tumor volume ~ 0.8-1.2 $cm^3$). Six groups with seven mice in each were formed. Samples of tumor, blood, skin and muscle were taken from anesthetic treated decapitated mice for subsequent analysis of gold content in 0.5, 1 and 3 h after compound administration.

For study of uniformity of the distribution of gold in the tumor volume, the samples of tumor were taken from anesthetic treated decapitated mice in 0.5, 1 and

3 h after compound administration. The samples were frozen and divided into four equal parts. Each part of the tumor was weighed and conducted the preparation of tissue samples for concentration analysis of gold.

The unbiased estimate of the mean ($\overline{X}$) and the unbiased standard deviation of the mean ($S\overline{x}$) were calculated for all experiments.

### 2.3 Preparation of tissue samples for concentration analysis of boron and gold.

Tissues samples were quantitatively transferred using distilled $H_2O$ into a quartz vessel (crucible, beaker) and dried in a drying oven at 80-105°C until completely dry. The dried samples were added with MgO (25 mg) (to reduce the loss of boron) and dropped with $H_2O$, then mixed and evaporated to dryness on a hot plate. The biological samples were decomposed by dry ashing in an EKPS-50 muffle furnace (Russia) in two steps. First, the samples were heated to 200°C and then to 500°C in steps of 50°C for 15 min each. Samples were held at this temperature for 5-6 h until a white or grayish-white ash was obtained. In a quartz vessel with ashed samples were added $H_2SO_4$ solution (5 ml, 1 N), mixed (2-3 min) and left for 10 min. The contents of the beakers were transferred to centrifuge tubes then distilled $H_2O$ (up to 10 ml) were added. The boron content in the samples was determined using an optical emission method with inductively coupled plasma (ICP-OES, Varian, Australia).

Preparation of biological samples for analysis of gold concentration was carried out by the described above method for boron with the difference that MgO was not added to the analyzed samples, but 1-2 ml of Aqua Regia was added in quartz beakers with ashed samples, then mixed before dissolution and dried until completely dry. 5 ml HCl (0.05 N) were added to the precipitate and left for 10 min.

## 3. Results

### 3.1 The study of boron compound biodistribution.

Figure 1 presents results related to the accumulation dynamics of the boron compound based on hyaluronic acid in tumor, blood, muscle and skin after the intratumoral administration. The maximal boron content in tumor (55 μg $g^{-1}$) was observed in 15 min after the administration, but it was twice less in 1 h and decreased 5 times in 3 h. This fact indicated that the compound was washed out rather quickly from tumor. Significant accumulation of the compound in the liver, kidneys, spleen, and lungs was not observed.

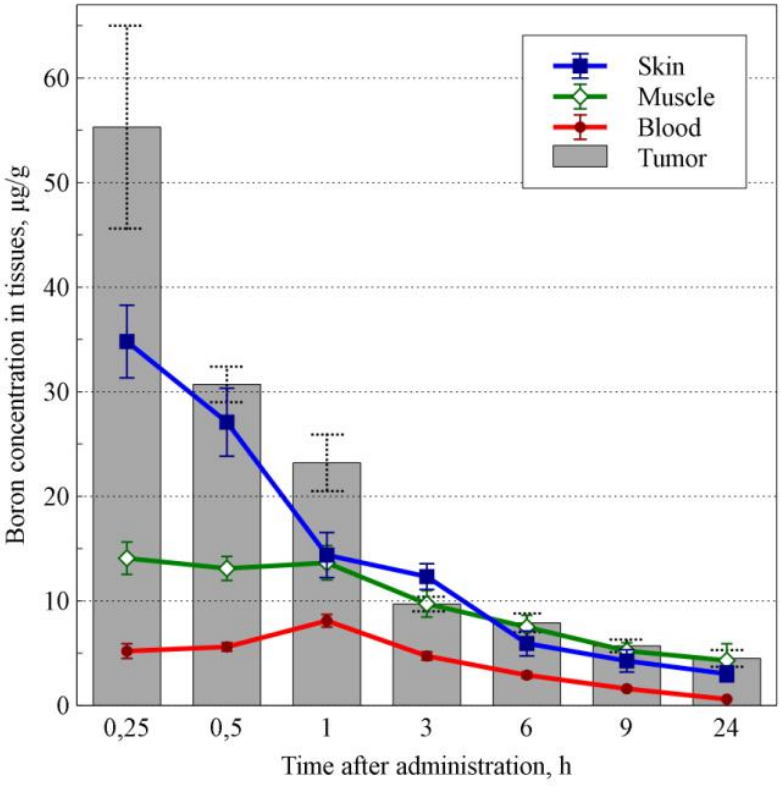


**Figure 1.** Boron concentration ($\overline{x} \pm S_{\overline{x}}$) in tumor (columns) and skin (square), muscle (diamond) and blood (circle) at various times after the intratumoral administration of boron compound Borhyal (3 mg $mL^{-1}$).

### 3.2 The study of gold compounds biodistribution.

Figures 2-3 show the results related to the accumulation of two gold compounds with hyaluronic acid and melanin (P-1 and P-2). Analysis of the data shows that in 30 min after compound administration, the gold concentrations in tumor were 180 μg $g^{-1}$ (for P-1) and 260 μg $g^{-1}$ (for P-2). Regardless of the fact that the gold concentration in tumor for P-1 was lower than P-2, the preparation P-1 showed better ability to be kept in tumor up to 3 h. At the same time the gold content in tumor for preparation P-2 compared with the data for 30 min was decreased two times in 1 h and 3.5 times in 3 h after the administration. The decrease of the content of preparations P-1 and P-2 in tumor showed marked decrease for preparation P-2 and was accompanied by increasing of the gold concentration in blood and skin (Figure 3). The greatest concentration values were achieved in 3 h after the administration of these preparations.

Because of a greater uptake of preparation P-1 in the tumor, better tumor/blood and tumor/skin

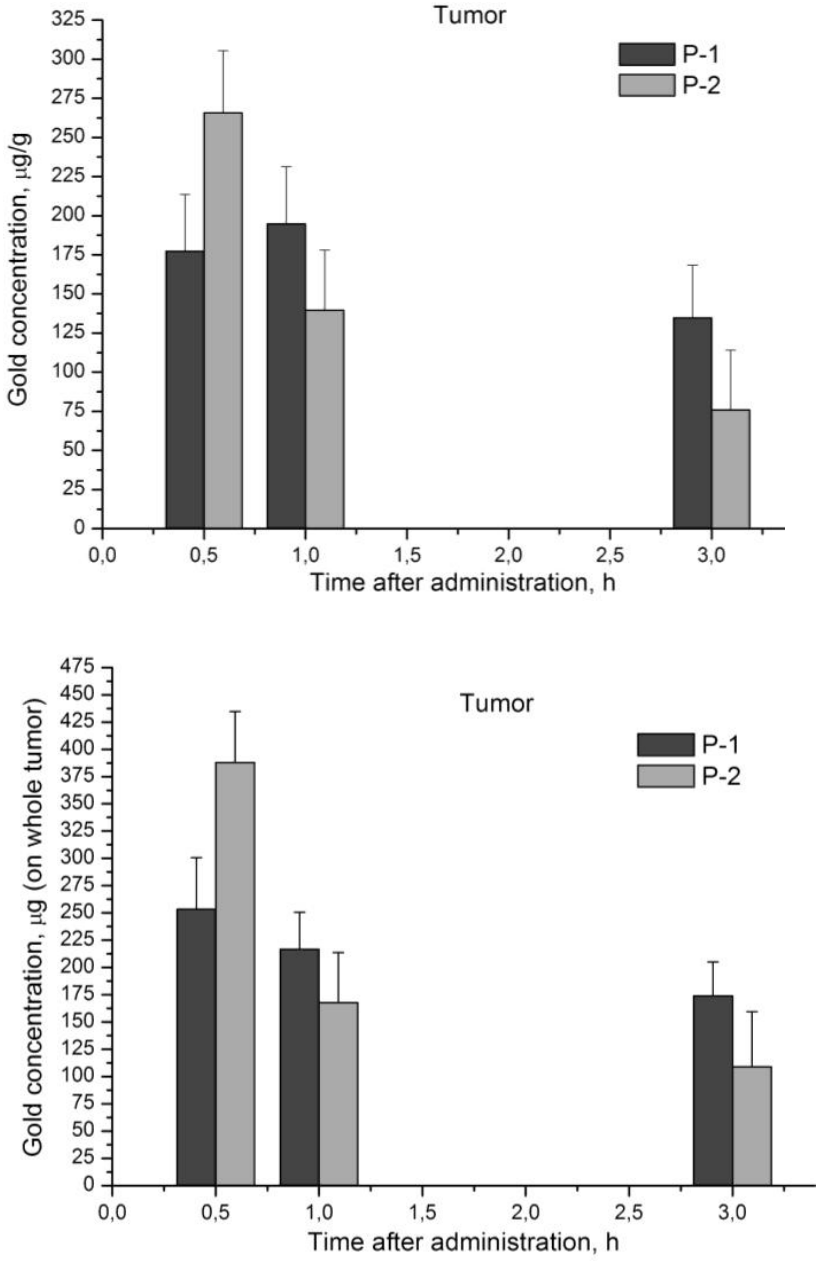


**Figure 2.** Distribution of the gold compounds based on hyaluronic acid in tumor (μg Au per 1 g of tumor (a) and absolute value of in tumor (μg Au; average tumor volume ~ 1 $cm^3$ (b)) at 0.5, 1 and 3 h after the intratumoral administration.

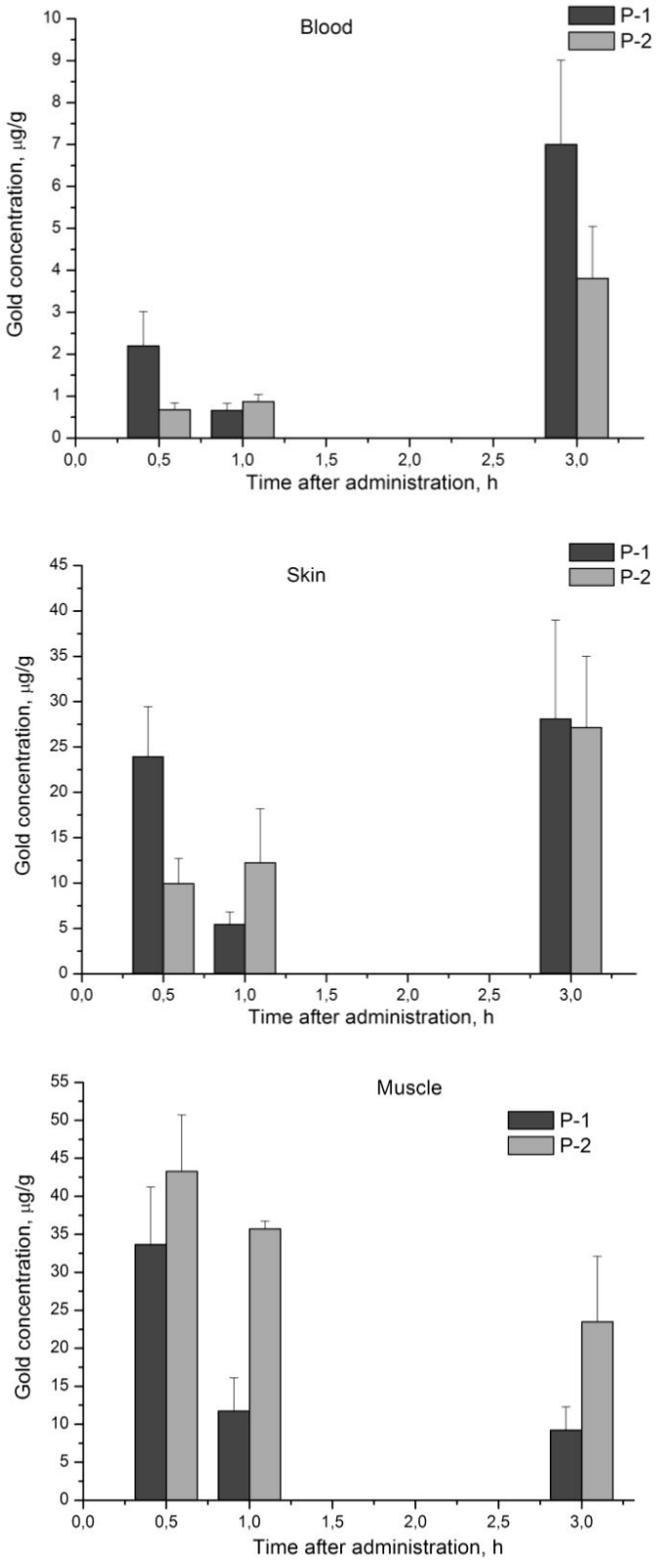


**Figure 3.** Distribution of the gold compounds based on hyaluronic acid in blood, skin and muscle at 0.5, 1 and 3 h after the intratumoral administration.

ratios (Figure 4) in 1 h after the administration were obtained.

### 3.3 The study of gold distribution at volume tumor.

The tumors were divided into four nearly equal parts for estimation of gold distribution uniformity in the tumor volume. Table 1 provides data on the percentage of gold in each of the parts. The result is presented as the mean and standard error.

Analysis of the results showed that the gold was unevenly distributed in the tumor volume. The zone of compound administration was expressed (maximum of gold content) contained about 50% of the gold. The uneven distribution of gold in the tumor volume remains for 3 h after the administration.

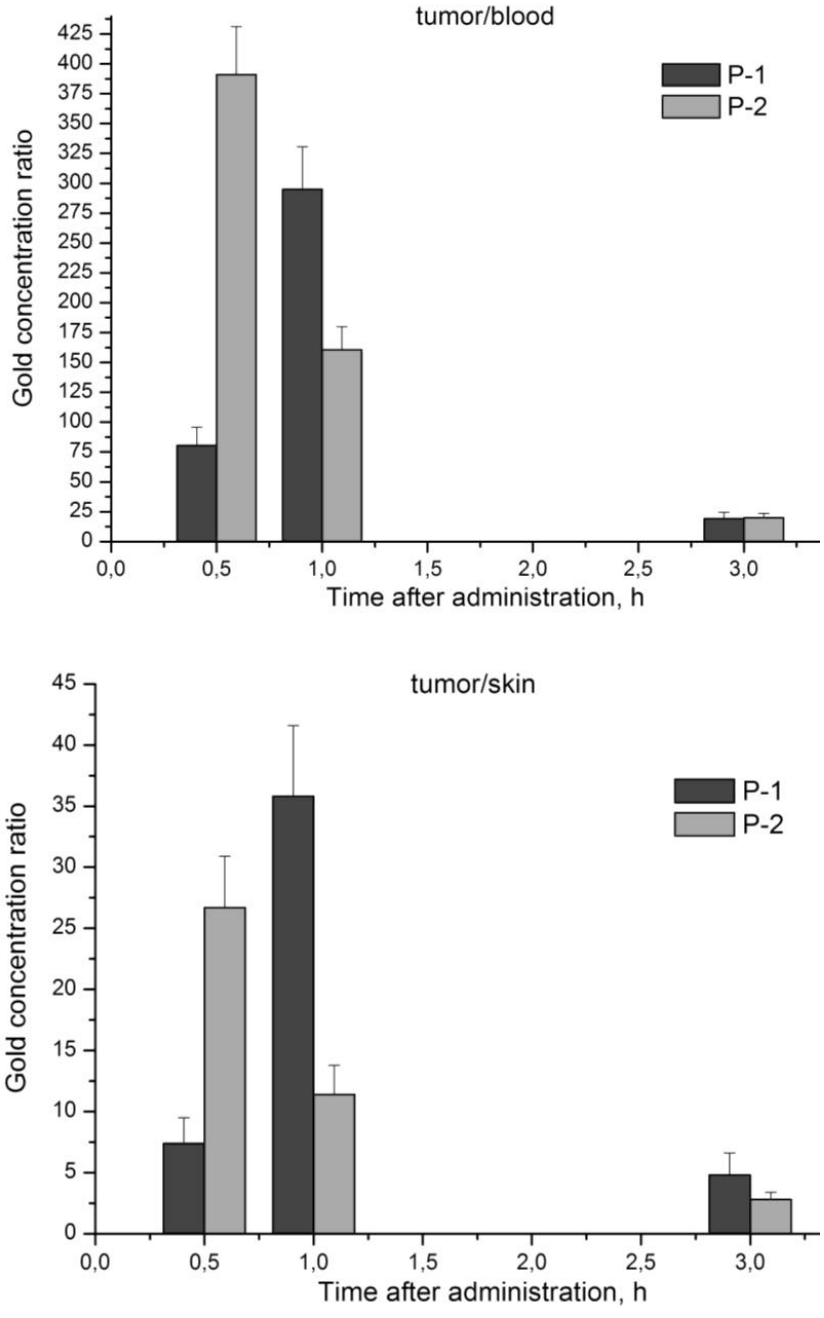


**Figure 4.** The ratios of gold content tumor/blood and tumor/skin at 0.5, 1 and 3 h after the intratumoral administration of P-1 and P-2 preparations.

| The parts of tumors | The percentage of gold in each of the parts | | | | | |
|---|---|---|---|---|---|---|
| | 0,5 h | | 1 h | | 3 h | |
| | P-1 | P-2 | P-1 | P-2 | P-1 | P-2 |
| 1 | 10.5±2.5 | 5.7±2.9 | 9.5±1.7 | 12.0±2.2 | 5.7±1.7 | 8.6±1.4 |
| 2 | 18.8±1.3 | 14.2±3.4 | 14.6± 3.3 | 18.2±2.0 | 14.2±1.7 | 16.3±2.5 |
| 3 | 28.2±2.6 | 27.0±2.9 | 28.7±2.5 | 23.5±1.7 | 24.6±2.2 | 25.8±3.3 |
| 4 | 42.6±4.1 | 53.1±7.0 | 47.0±4.1 | 46.3±3.5 | 55.6±2.3 | 49.3±5.4 |

**Table 1.** The percentage of gold in each of the parts of tumors at 0.5, 1 and 3 h after the intratumoral administration of P-1 and P-2 preparations.

## 4. Discussion

As it was mentioned above, the basic principles of NCT and PAT are similar. The value of the additional dose due to the injection may be significant and depends on the concentration of a capture/activation agent in a tumor tissue. The main condition of effective implementation of BNCT is high accumulation of $^{10}B$ in the tumor (20-30 mg per 1 g of tumor) with significant tumor/surrounding tissue ratio (greater than 1) [2]. In our studies the maximal content of $^{10}B$ in the tumor in 15 min after the administration (55 mg $g^{-1}$) and tumor/muscle and tumor/blood ratios (respectively 3 and 5) met the requirements. No significant accumulation of the compound were found in the liver, kidneys, spleen and lungs (indicating the fast output from the body of tumor-bearing animals) was observed. It was noticed that $^{10}B$ concentration in the tumor decreases rapidly with time. In 3 h after the administration its concentration in tumor became comparable to the accumulation in the surrounding tissues. Most probably it indicates the presence of free $^{10}B$ atoms uncombined with hyaluronic acid.

The noncytotoxic, nonimmunogenic, and biocompatible properties of gold nanoparticles are important issues for the potential application in medicine. Hainfeld J.J. *et al.* [8] presented data on gold nanoparticles accumulation in tumour, blood, and muscles of mammary carcinoma bearing mice in 5 min after intravenous administration (1.35 g per 1 kg of body weight). Gold concentration in tumor was determined to be 1.3 mg $g^{-1}$, the values in kidneys, blood, and muscles were 35.6, 5.0 and 0.4 mg $g^{-1}$, respectively. High gold nanoparticles content in blood and kidneys is evidence of their poor tumor-seeking capacity. Therefore, new gold carrier compounds are developed to selectively deliver gold into tumor cells in order to decrease total body gold burden. The choice of hyaluronic acid (HA) as gold carrier was due to its ability to recognize specific cellular receptors (CD44 and RHAMM) which are over expressed on the surface of various tumor cells. However, the concentration gold particles in our studies in the tumor tissue was approximately 20-30 times lower than required for effective realization of photon activation enhancement of radiotherapy. At the same time in some other study [8], good results were received. Before the exposure to X-rays mammary carcinoma bearing mice were intravenously injected with 1.9 nm diameter gold particles (2.7 g per 1 kg of body weight). In one month nine of ten mice that received gold particles shortly before the X-ray exposure (30 Gy) had no visible tumors. The tenth mouse had a shrinking tumor. At the same time, five-fold increase in tumor volumes (compared to its initial sizes) in case of mice receiving only X-ray therapy was observed. Concentration of gold in tumors was about 7 mg $g^{-1}$. Tumor/normal tissue concentration ratios had remained 8:1 during several minutes of X-ray therapy. Compared to that experiment we obtained gold concentration in the tumor about 0.2 mg $g^{-1}$. To enhance the gold content in the tumor tissue it is necessary to increase the amount in the initial compound. However, the method of synthesis suggests that the increase of gold atoms amount enhances the content of hyaluronic acid. It results in increased viscosity much higher than the blood viscosity of healthy humans or animals. High viscosity is an obstacle for administration of such compounds in the bloodstream and for its equal distribution in the tumor.

The difference in accumulation of compounds P1 and P2 (the accumulation in tumour for compound P2 in 1.5 times more in 30 min after administration) possibly due to more number of free functional groups on the polymer and as a consequence with the higher selectivity polymer.

The results of our study showed that there is considerable unevenness of gold distribution in the tumor volume. So for compounds P1 and P2, unevenness was 55% and 83%, 67% and 60%, 44% and 70.8% correspondingly in 0.5, 1 and 3 h after compound administration. There was not a high uniformity of the distribution of gold in the tumor volume in our study and study report [8]. In the case of X-ray irradiation of the tumor (in the course of photon activation therapy)

such a uniform distribution of gold in the tumor makes it difficult to calculate the isodose distribution by the volume. There are both overexposure areas (permitted in radiotherapy) and underexposure areas (not acceptable since the remaining live tumor cells grow results in the relapse). The solution here is to introduce the preparation in different areas tumor or around the tumor. Another way is to decrease preparation viscosity.

On the assumption of the above, the investigated gold compounds in its present form cannot be used in photon activation therapy. At the same time they can be used for diagnostic purposes, using such a property of hyaluronic acid molecule as a tropism for tumor cell surface receptors. Also in this case, the concentration of gold atoms in the tumor tissues is not such an important factor.

## 5. Conclusion

Results of studies of the new Russian compound based on hyaluronic acid and boron showed that its uptake in tumor was highest (sufficient for neutron capture therapy) during the first hour after the intratumoral administration. The most appropriate decision in this case is to introduce this compound 15-30 min before the neutron irradiation when the boron concentration in tumor is higher than in surrounding tissues.

The study of distribution of two gold compounds with hyaluronic acid in 0.5-3 h after a single injection shows that the maximal concentration of gold in tumor was in 30 min (180-260 μg $g^{-1}$) after the intratumoral administration. It is approximately 8-15% of injected amount and, unfortunately, 20 times less than required for effective application of the photon activation therapy. At the same time the high ratios of the gold in tumor and surrounding tissues obtained in 0.5 and 1 h after the administration should be noted. High value of this ratio is an important and necessary condition to form the local absorbed dose in tumor during PAT. The gold was unevenly distributed in the tumor volume on the whole interval of research. The solution of this problem is to use the method of compound administration in different zones of the tumor: the injections around the tumor, or the introduction in the artery supplying the tumor.

In general, on the basis of obtained data it is possible to assume the perspectives of these non-toxic compounds with hyaluronic acid for use in boron neutron capture therapy, and the gold compounds for use primarily as a contrast agent for diagnostic purposes.

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