Radiopharmaceutical development based on human blood albumin microspheres and 90Y

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Abstract. New radiopharmaceutical (RP) based on human serum albumin microspheres (MSA) and 90Y was developed for treatment of liver cancer. The optimized synthesis using chelation resulted in approximately 80% yield with high specific activity. The RP developed was tested in mice with inoculated sarcoma-37. In two weeks the tumor size reduced by 43% after the treatment with the dose of 500 µCi injected into the tumor site.

1. Introduction
The current investigation is focused on the design of the new radiopharmaceuticals (RP) for the treatment of oncology. Cancer research is socially important especially keeping in mind the increase of malignant tumor diagnosis in the last years [1].

Oncological diseases remain one of the main cause of death in the developed countries. In Russia 280,000 men die from cancer each year. Cancer is responsible for the 15.3% of all mortality in the country next to cardiovascular diseases (50.1% in 2014) being ahead of trauma and poisoning (8.0% in 2014). 80641 men died in the working age 15-59 years as a result of cancer (15.4% for this group).

Cancer database of the P. Hertsen Moscow Oncology Research Institute reports that the number of primary liver cancer diagnosis changed from 6570 in 2004 to 7252 in 2014 with 10% increase.

Liver cancer can be primary namely originating from the liver cells themselves and secondary (metastatic) which is characterized by the growth of the tumor cells originated in the other organ and transported later to the liver [3].

Metastatic liver cancer is observed much more frequently than the primary one because of the liver function in the body and because of the high vascularization and perfusion.

Patients with the primary cancer of intestine, pancreas, stomach, lungs and reproductive system are prone to develop liver metastasis. Generally liver metastases are developed in 1/3 of all cancer patients. Metastatic cancer is characterized by the fast progression with no specific clinical observations [3]. The prognosis is negative predicting survival rate of 50% after one year [1].

Therefore the treatment of the liver malignant tumor remains of a key importance in clinical oncology. Cancer treatment depends on the stage of the disease and it can include surgery,
chemotherapy and radiotherapy. Still the best results that allow the long survival time are achieved with the surgery. But at the time of diagnosis the radical resection of the tumor is possible with 5-15% of the patients only. Recurrent tumor is observed with 70-90% of the patients in 3-5 years. The second resection is possible with no more than 10% of the subjects. Hence the non-surgical treatment is of a vital importance for more than 80% of the liver cancer patients [4].

Currently in case of no surgery possible the systemic chemotherapy is used. The alternative is the locoregional chemo infusion and chemoembolization of the liver artery.

Last years the new approach of arterial radioembolization (RE) – a combination of the embolization and the radiotherapy was suggested. The method is based on the microspheres filled with isotope $^{90}$Y (Theraspheres, MDS Nordion, Canada) or polymer microspheres again with $^{90}$Y (SIR-spheres, Sirtex Medical, Australia) that are injected in the liver artery. Therapeutic dose is achieved in two weeks post injection [5,6].

RE implementation demonstrates good prospective of the method. However up to now there were only 6 patients in Russia treated with RE. Glass microspheres doped with $^{90}$Y were used in 2009 in Russian Research Center for Radiology and Surgical Technologies (St.-Petersburg) with 4 patients. In 2012 another 2 patients received RE at Russian Cancer Research Center (Moscow). The limiting factor remains the high price of the procedure and the short lifetime of $^{90}$Y [6] especially taking into account the transportation time to Russia.

Development of a new highly efficient radiopharmaceutical is a multidisciplinary task with two main backbones: a selection of a proper radionuclide and a carrier among which human serum albumin plays an important role [7].

Microspheres of human serum albumin (MSA) are the unique carriers for the selective delivery of a radionuclide to the malignant tumors. Radiopharmaceuticals based on human serum albumin are highly physiological and easy to produce. The micro particles can be created with the controlled size and a given proteolysis speed in the body, ability to incorporate almost any isotope, keep them bound and release simultaneously with the proteolysis of the denatured protein [8–10].

The goal of the current study is to develop a radiopharmaceutical based on HBA and $^{90}$Y to ally for the primary and metastatic liver cancer.

2. Materials and methods
The study on optimal fixation of $^{90}$Y with human blood albumin microspheres 25-40 µm in diameter both non-modified and modified by the chelator were performed in the laboratory of Experimental Nuclear Medicine at A.Tsyb Medical Radiological Research Center – a branch of The Federal State Budgetary Institution the National Medical Research Radiological Center of the Ministry of Health of the Russian Federation. (FSBI NMRRC)

The following reaction conditions were studied: the acidity of the reaction mixture, heating time and the reaction temperature, influence of the other metals impurity on the fixation rate with $^{90}$Y.

The results of $^{90}$Y and MSA binding (both chelator modified and non-modified) are presented in table 1. It is clear modified MSA binds $^{90}$Y two times better than non-modified one. This result is confirmed by serial experiments. Therefor only modified MSA was taken for further experiments.

After the extra purification of the metals impurity from the $^{90}$Y solution the fixation rate of $^{90}$Y with MSA increased up to 83% (the second and the third preparations). The fixation rate of $^{90}$Y with MSA depends on the amount of inactive impurities of Fe, Al and Zn and we suppose that $^{90}$Y chloride solution of a high quality will bind to MSA with 95% rate which is a good prerequisite for the development of a “cold kits” of radiopharmaceuticals for the liver cancer therapy.
Table 1. Fraction of $^{90}$Y – MSA binding in %.

| Substance                  | 0,1 | 1   | 2   | 24  |
|---------------------------|-----|-----|-----|-----|
| First preparation of $^{90}$Y chloride |     |     |     |     |
| $^{90}$Y-MSA               | 23  | 26  | 30  | 30  |
| $^{90}$Y-chelator-MSA      | 43  | 69  | 65  | 84  |
| Second preparation of $^{90}$Y chloride |     |     |     |     |
| $^{90}$Y- MSA              | 39  | 42  | 43  | 38  |
| $^{90}$Y- chelator-MSA     | 76  | 81  | 82  | 80  |
| Third preparation of $^{90}$Y chloride |     |     |     |     |
| $^{90}$Y- chelator-MSA     | 68  | 83  | 70  | 75  |

The therapeutic efficacy on mice with inoculated tumor was also studied at FSBI NMRRC (figure1). The study was performed on female laboratory mice (N=30) divided into three groups 10 animals each. All mice were inoculated with sarcoma-37. In order to obtain a solid tumor the ascites fluid taken from donor mice was transplanted at day 8–10 of the tumor growth. The suspension of the tumor cells was injected under the hip skin. The study of the therapeutic efficacy started at day when the tumor size reached 0,8 – 1,0 cm³.

![Figure 1](image1.png)

*Figure 1*. The study of the therapeutic efficiency of the designed radiopharmaceutical.
The first and the second groups received $^{90}$Y-chelator-MSA 9.25 MBq (250 µCi) and 18.5 MBq (500 µCi) respectively injected in the tumor. The third group received the physiological solution injection of the same volume and served as control. The tumor volume was measured in all groups during 14 days next to transplant. All data in between was interpolated.

3. Results
In this experiment an active radiopharmaceutical was obtained with the yield of 82%. The specific volume activity was 126 mCi/ml which is high enough for the clinical applications.

The synthesis of MSA both chelated and pure was efficiently optimized to achieve the required characteristics.

The study of the therapeutic effect of the developed drug revealed the maximum retardation of the cancer growth achieved on day 14. compound compared to the control group (figure 2).

![Figure 2](image-url)

**Figure 2.** The retardation of a cancer growth (sarcoma-37) in mice after intratumor injection of $^{90}$Y-albumin microspheres with the diameter of 25–40 µm.

4. Conclusions
The conducted radiochemical and biological studies concludes that $^{90}$Y-albumin microspheres with the diameter of 25–40 µm is a prospective radiopharmaceutical for malignant tumors.

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