Study of Peptide Fragment of Alpha-Fetoprotein as a Radionuclide Vehicle

Abstract—Alpha-fetoprotein and its fragments may be an important vehicle for targeted delivery of radionuclides to the tumor. We investigated the effect of conditions on the labeling of biologically active synthetic peptide based on the (F-afp) with technetium-99m. The influence of the nature of the buffer solution, pH, concentration of reductant, concentration of the peptide and the reaction temperature on the yield of labeling was examined. As a result, the following optimal conditions for labeling of (F-afp) are found: pH 8.5 (phosphate and bicarbonate buffers) and pH from 1.7 to 7.0 (citrate buffer). The reaction proceeds with sufficient yield at room temperature for 30 min at the concentration of SnCl\textsubscript{2} and (F-afp) is to be less than 10 mkg/ml and 25 mkg/ml, respectively. Investigations of the test drug accumulation in the tumor cells of human breast cancer were carried out. Results can be assumed that the in vivo study of the (F-afp) in experimental tumor lesions will show concentrations sufficient for imaging these lesions by SPECT.

Keywords—peptide, technetium-99m, tumor, SPECT.

I. INTRODUCTION
ALPHA-FETO PROTEIN or its fragments are promising agents for the tumors imaging. Alpha-fetoprotein is not produced in the adult healthy organism, as well as it receptors. At the same time, the presence of receptors for alpha-fetoprotein is typical for many types of tumor cells. Since the AFP itself quite expensive and difficult to reach, it seems reasonable to use the fragments of its molecule, especially those who are responsible for its biological behavior.

Peptide used in this study is the fragment of the (F-afp), house responsible for binding to receptors on the cell surface. Proteins such as alpha-fetoprotein, are involved in the regulation of tissue growth, formation and development of body parts and body systems, including nervous, cardiovascular, reproductive, etc. [1-3].

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II. MATERIALS AND METHODS
A. Obtaining of the Tc-99m solution
The solution of Tc-99m as pertechnetate ion in isotonic sodium chloride solution was derived from the generator GT-2M or CRS-3-FHI (Russia) according to manufacturer's specifications.
instructions.

B. The reaction of the peptide labeling

Sample (1 mg) of the lyophilized peptide fragment was dissolved in 1 ml of distilled water, packed up to 30 ml in Eppendorf tubes and frozen. It was kept frozen at -18°C for 2-3 weeks.

A sample of peptide was kept at room temperature for 5-10 minutes. Then we added a buffer solution and the solution of tin chloride (II) in argon atmosphere. The $^{99m}$TcSO$_4$ solution was added to this mixture. The reaction mixture was incubated at room or higher (up to 70°C) temperature.

C. Analysis of the reaction medium

To determine the unreacted pertechnetate TLC silica gel (Merck 5553) was used. Pertechnetate is eluted with acetone or methyl ethyl ketone (Rf 0.9-1.0). To determine the labeled peptide TLC on cellulose plates (Merck 5552) was used. The labeled peptide is eluted with a mixture of acetonitrile/water at a ratio of 1:1 (Rf 0.9-1.0). Analysis methods were also tested using other solid phases and solvent systems. Radiochemical yield of the labeling was calculated based on the data obtained in both assays.

D. Study of the dynamics of the total accumulation of $^{99m}$Tc-(F-afp)

To characterize the biological behavior of $^{99m}$Tc-(F-afp) in vitro their affinity to the structures of cell line MCF 7 on indicators of total accumulation and the strength of binding (externalization) was investigated.

100 ml of the test drug were brought to tubes containing cell MCF 7 suspension:
- the concentration of active peptide fragment of AFP 20 mg/ml;
- volume activity - 1 mCi/ml $^{99m}$Tc.

The tubes were placed in an incubator at 37°C and incubated for 15, 30, 60 and 90 minutes. Accumulation of labeled compounds was stopped by placing tubes to the ice for short time. Then the cells were separated by centrifugation, the radioactive solution was removed and the cells were washed with 3-fold volume of cooled Hanks’ solution followed by centrifugation and removal of supernatant. Activity accumulated by cells was measured by direct radiometry. Accumulated cell activity was normalized as a percentage of the activity introduced in 10⁶ cells.

E. Investigation of the binding strength of $^{99m}$Tc-(F-afp) with tumor cells

Investigation of the binding strength of $^{99m}$Tc-(F-afp) with tumor cells was performed to indirectly assess of the functional suitability of these compounds in vivo. For this purpose conditions were simulated living organism, i.e., cells were incubated in a medium whose composition close to the composition of blood plasma, incubation temperature was 36.8°C. The binding strength was studied by washing-out. To maximize the exception of cell damage during the handling them, the experiment was carried out with monolayers of cell culture. To culture vials containing a complete monolayer of cells and 5 ml of culture medium 200 ml of $^{99m}$Tc-(F-afp) was added. Cells were incubated for up to a maximum accumulation of the studied compounds. Then the radioactive solution was removed, the monolayer was gently washed-out three times with Hanks solution and applied to 5 ml of fresh medium. The level of accumulated cell activity was measured by direct radiometry. Then the culture flasks were placed back into an incubator and reincubation procedure was started. During this process a sample ( aliquot) was taken of the culture medium at certain intervals. Aliquots of medium were also subjected to radiometry. At the end the dynamics of washing-out of radioactive products from the cells into the environment was determined.

Radiometry of obtained samples was carried out on an automatic γ-counter Wizard 2480 ("PerkiElmer LAS / Wallac", Finland).

In all in vitro studies $^{99m}$Tc-(F-afp), prepared using citrate buffer, was used.

III. RESULTS

A. Choice of labeling conditions

The influence of the buffer mixture nature on the efficiency of labeling was investigated using phosphate, bicarbonate and citrate buffers. Results of labeling in phosphate buffer are shown in Figure 2. The figure shows that the labeling occurs with a fairly good yield only at pH above 8.5.

Similar results were obtained when labeling a bicarbonate buffer solution at pH from 8.4 to 10.5. At the same time, using of citrate buffer allows to label (F-afp) under acidic conditions, in the pH range from 1.7 to 7.0.

Data on the effect of tin quantity on the influence on labeling are shown in Figure 3. It is evident that a wide range of concentrations of tin dichloride has no appreciable effect on the yield of the labeling. By reducing the concentration of tin (II) increases the proportion of pertechnetate ion, whereas at high concentrations of tin - hydrolyzed reduced technetium.
Fig. 3 Dependence of the labeling reaction yield on the amount of reducing agent in the reaction medium (pH 8.8)

The influence of the concentration of peptide on the labeling efficiency was also studied (Fig. 4).

Fig. 4 Dependence of the labeling reaction yield on the amount of reducing agent in the reaction medium with citrate buffer

B. Study of the dynamics of the total accumulation of $^{99m}$Tc-(F-afp) in breast adenocarcinoma cells MCF-7

The ability of $^{99m}$Tc-(F-afp) to bind to tumors that express receptors for alpha-fetoprotein is shown by results of in vitro experiments. The data obtained clearly show the ability of the studied radiopharmaceutical to accumulate in the cells of human breast adenocarcinoma MCA 7. In order to assess the influence of possible impurities on the level of binding ions of unreacted technetium-99m, the generator $^{99}$Mo/$^{99m}$Tc eluate was used as the reference solution. The experimental results are presented in Figure 5.

Fig. 5 Dynamics of accumulation of $^{99m}$Tc-(F-afp) by the cells of human breast adenocarcinoma MCF-7

As can be seen, the behavior of the compound under in vitro study is characterized by its affinity for cells of adenocarcinoma of the breast. Already after 60 min accumulation of $^{99m}$Tc-(F-afp) reaches its maximum value, a few more than 4% of activity introduced in $10^6$ cells, and remains stable for at least another 30 minutes, which allows to include the possibility of visualizing of malignant neoplasms lesions with SPECT studies. As expected, the accumulation of $^{99m}$Tc-citrate was significantly lower than that in the complexes under study. Thus, it is proved that the presence of citrate ions in the reaction mixture and the probable formation of a certain amount of $^{99m}$Tc-citrate does not determine the levels of accumulation of $^{99m}$Tc-(F-afp) observed.

In the second series of experiments, we compared the levels and nature of accumulation of different lines human malignancies cells, namely MCF 7 and A 549, compared with normal. The control lines were used human lung fibroblasts (HLF). Figure 6 shows the data obtained.

Fig. 6 Dynamics of $^{99m}$Tc-(F-afp) accumulation in human cells of different lines

The findings prove $^{99m}$Tc-(F-afp) specific accumulation in tumor, which suggests the possibility of diagnosing malignant tumors of different origin and localization.
C. Estimation of strength of binding of $^{99m}$Tc-(F-afp) with tumor cells.

The dynamics of radioactive products washing-out from the cells of breast adenocarcinoma MCF 7, human lung carcinoma A 549 to the culture medium is shown in Figure 7.

![Fig. 7 Dynamics of radioactive products washing-out from tumor cells](image)

In this experiment, we registered only the appearance of the radioactive isotope $^{99m}$Tc in the culture medium, without identifying those compounds it was associated with. As can be seen from Figure 7 the washing-out processes from cells MCF 7 and A 549 have different dynamics. The experimental results confirm that the active peptide fragment of $^{99m}$Tc-(F-afp), being internalized by cells of human breast adenocarcinoma, degraded with the release of metabolic products relatively slowly, as evidenced by its strong retention of activity in cells. Probably because of the appearance of radioactive products in the culture medium during the reincubation procedure, it is responsible that part of the active peptide fragment, which was not internalized, and got associated with membrane receptors. Human lung carcinoma cells did not show an equally strong binding of $^{99m}$Tc-(F-afp) and their structures, which may be due to low density or even absence of appropriate receptors for alpha-fetoprotein in their nuclear membranes.

IV. CONCLUSION

The conditions for labeling of bioactive synthetic peptide based on the (F-afp) by technetium-99m have been studied. As a result of this work, the following optimal conditions for labeling of (F-afp) are obtained: a pH 8.5 (phosphate and bicarbonate buffers) and pH from 1.7 to 7.0 (citrate buffer). The reaction proceeds with sufficient labeling yield at room temperature for 30 min at the concentration of SnCl$_2$ and (F-afp) in the reaction mixture not less than 10 mg/ml and 25 mg/ml, respectively.

An investigation of receptor specificity of the test radiopharmaceutical was carrying out on cells of human breast cancer, which have receptors for alpha-fetoprotein at its membrane. These results make us assumed that when studying the biological behavior of the compounds studied in vivo in experimental tumor lesions will achieve concentrations sufficient to visualize these lesions by SPECT.

REFERENCES

