

Synthesis and In Vitro Evaluation of $Tc(CO)_3^+$ Complexes with Dithiocarbamate Ligands Containing Morpholine Moiety

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The aim of this work was to synthesize and study complexes of $M(CO)_3^+$ with dithiocarbamate ligands included morpholine moiety which have high affinity to melanoma. The corresponding complexes of rhenium (A and B) were obtained by simultaneous mixing of bidentate (dithiocarbamate) and monodentate (isonitrile) ligands with $[Re(CO)_3Br_3][NEt_4]_2$. For the comparative evaluation of these technetium-99m complexes for diagnostics of melanoma the study of affinity of these complexes to the cells of melanoma B 16 in vitro was carried out.

1. Introduction

During the last ten years a lot of technetium and rhenium complexes with mixed ligands included $M(CO)_3^+$ group was studied and described in the literature. It is due to the development of new radiopharmaceuticals and first of all with radiopharmaceuticals for tumor investigations.

It was found earlier^{1,2} that dithiocarbamates react with $M(CO)_3(H_2O)_3^+$ to form complexes extremely stable for a long period in both basic and acidic medium.

The aim of this work was to synthesize and study complexes of $Tc(CO)_3^+$ (^{99m}Tc-TC) with dithiocarbamate ligands included morpholine moiety which have high affinity to melanoma.³

2. Material and Methods

The technetium complexes (^{99m}Tc-TC-I, ^{99m}Tc-TC-II, ^{99m}Tc-TC-III, ^{99m}Tc-TC-IV, ^{99m}Tc-TC-V, ^{99m}Tc-TC-VI) were obtained in two steps. Firstly the solution of ^{99m}Tc(CO)₃(H₂O)₃⁺ with pH 7 was obtained from "IsoLink" kit. Then different ligands [propyl-morpholin-dithiocarbamate (L₁), morpholin-dithiocarbamate (L₂), isocyanoacetic acid (L₃), tret-butyl-isonitril (L₄), nitrozy-sulfuric acid (L₅), diethyl-dithiocarbamate (L₆), ethyl-isocyanoacetate (L₇) and propyl-morpholin-isonitril (L₈)] were added to the solution. The ligand concentration in the mixture was 1.5×10^{-3} M. The reaction mixture was heated in the water boiling bath during 20 min. After cooling the solution to room temperature pH was adjusted to 7 with 0.1 M HCl. The reaction mixture was analyzed using TLC on silica gel in MeOH/HCl conc. (99/1).

complex	Ligand in position A	Ligand in position B
^{99m} Tc-TC-I	L ₄	L ₁
^{99m} Tc-TC-II	L ₅	L ₆
^{99m} Tc-TC-III	L ₃	L ₂
^{99m} Tc-TC-IV	L ₇	L ₁
^{99m} Tc-TC-V	L ₃	L ₁
^{99m} Tc-TC-VI	L ₈	L ₆

For the cell culture and cell uptake study the breast adeno-

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carcinoma MCF-7 and melanoma B 16 cell lines were grown in monolayers in RPMI 1640 medium, supplemented with 10% fetal bovine serum, amino acids, vitamins and penicillin-streptomycin, in humidified 5% CO₂/air atmosphere at 37 °C. Cell growth status was monitored by inverted microscope with phase contrast, and cell viability was assessed by trypan blue exclusion. For all experiments, the cells were seeded at density of 3 million cells/ flask 25 cm². Before the experiments the medium was changed to RPMI 1640 without serum. Then the needed amount of radiopharmaceuticals was added to the cell culture and the cells were incubated at 37 °C. After various incubation times (30, 60, 90, 120, 180 min) cellular uptake was stopped by removing medium from cells and washing twice with 5 mL phosphate-buffered saline (PBS). The cell uptake was calculated as percentage of the activity counted in the flasks containing cells (after subtracting the absorbed activity) relative to the total activity (in the flasks containing cells and medium). The cell uptake was presented in percentage per 1 million of cells.

The efflux of radioactivity from the cells was determined in experiment with ^{99m}Tc(CO)₃Cit and TC-^{99m}Tc-VI (containing L₂ and L₈) in both cell lines. The cells were incubated for 90 min to allow cell uptake of tracers to reach a maximal level. The radioactive medium was then removed, the cells were washed twice with 5 mL of PBS and then 5 mL of fresh RPMI 1640 was added to the cells for re-incubation. The cell uptake was determined once again at the end of re-incubation. The cell efflux was calculated as difference of the total activity (in the flasks containing cells and medium) and activity in the flasks containing cells (after subtracting the absorbed activity).

3. Results and Discussion

For the comparative evaluation of synthesized technetium-99m complexes for diagnostics of melanoma the study of affinity of these complexes to the cells of melanoma B 16 and breast adenocarcinoma MCF 7 in vitro was carried out. ^{99m}Tc(CO)₃Cit, which was earlier described⁴, was used as a comparative radiopharmaceutical.

The uptake of ^{99m}TcO₄⁻ (0.2%/million) was significantly lower than that of ^{99m}Tc(CO)₃Cit, ^{99m}Tc-TC-I, ^{99m}Tc-TC-II, ^{99m}Tc-TC-III, ^{99m}Tc-TC-IV, ^{99m}Tc-TC-V, ^{99m}Tc-TC-VI in all cells. So we can conclude that technetium-99m in the form of pertechnetate-ions does not bind with cells.

The kinetics of cellular uptake of ^{99m}Tc-TC-I, ^{99m}Tc-TC-II,

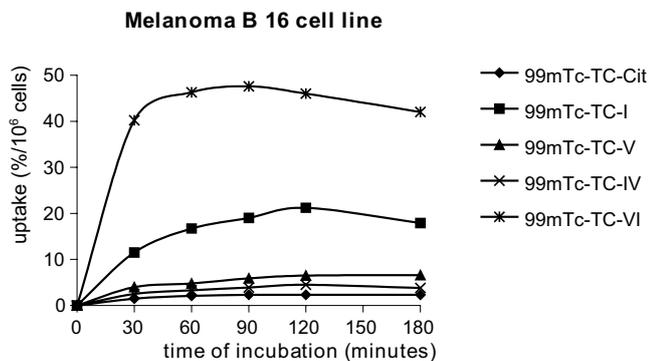


Figure 1. Uptake of ^{99m}Tc -complexes in cell lines.

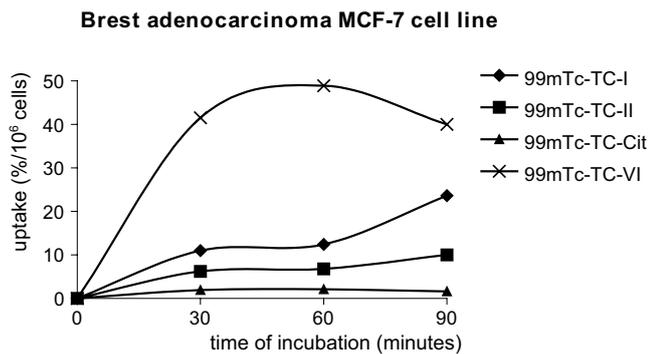


Figure 2. Uptake of ^{99m}Tc -complexes in cell lines.

$^{99m}\text{Tc-TC-VI}$ and $^{99m}\text{Tc}(\text{CO})_3\text{Cit}$ in breast adenocarcinoma MCF 7 cell line was shown in Figure 1. The kinetics of cellular uptake of $^{99m}\text{Tc-TC-I}$, $^{99m}\text{Tc-TC-IV}$, $^{99m}\text{Tc-TC-V}$, $^{99m}\text{Tc-TC-VI}$ and $^{99m}\text{Tc}(\text{CO})_3\text{Cit}$ in melanoma B 16 cell line was shown in Figure 2.

The maximal accumulation in the cells was observed after 90–120 min from the beginning of incubation. The accumulation of $^{99m}\text{Tc}(\text{CO})_3\text{Cit}$ does not exceed 2.3%/million in both cell lines. The highest uptake in both cell lines was for $^{99m}\text{Tc-TC-VI}$ (about 50%/million). The affinity of the other complexes were in several times lower and decreases in the following sequence $^{99m}\text{Tc-TC-VI} > ^{99m}\text{Tc-TC-I} >> ^{99m}\text{Tc-TC-II} > ^{99m}\text{Tc-TC-Cit}$ (breast adenocarcinoma MCF 7 cell line) and $^{99m}\text{Tc-TC-VI} > ^{99m}\text{Tc-TC-I} >> ^{99m}\text{Tc-TC-V} > ^{99m}\text{Tc-TC-IV} > ^{99m}\text{Tc-TC-Cit}$ (melanoma B 16 cell line). It is very difficult to explain why the complex $^{99m}\text{Tc-TC-VI}$ possesses the highest affinity to melanoma cells, because the data of its biological behavior in vivo does not coincide with data in vitro. The accumulation in tumor in melanoma bearing-mice is about 1.8%/g after 1 h post injection. This fact we can explain, if we assume, that this complex has a very high lipophilicity in comparison with other complexes because its accumulation in the intestine is about 68% after 1 h post injection. It is the highest accumulation in the intestine in comparison with other complexes. So the mechanism of cell uptake can be the following—more lipophilic complex easier penetrates through the cell membrane and accumulates inside the cell.

Due to the highest cell uptake $^{99m}\text{Tc-TC-VI}$ was used for cell efflux study. It is shown that cell efflux does not exceed 30% after 1.5 h re-incubation for both complexes

($^{99m}\text{Tc}(\text{CO})_3\text{Cit}$ and $^{99m}\text{Tc-TC-VI}$) in both cell lines. Figure 3 shows the percentage of cell activity after 1.5 h re-incubation.

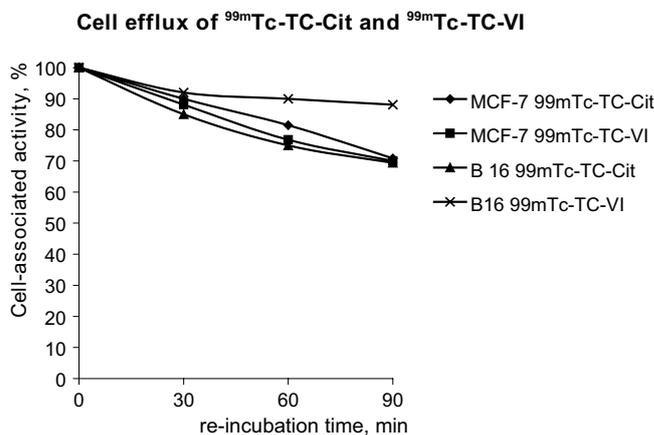


Figure 3. Cell efflux.

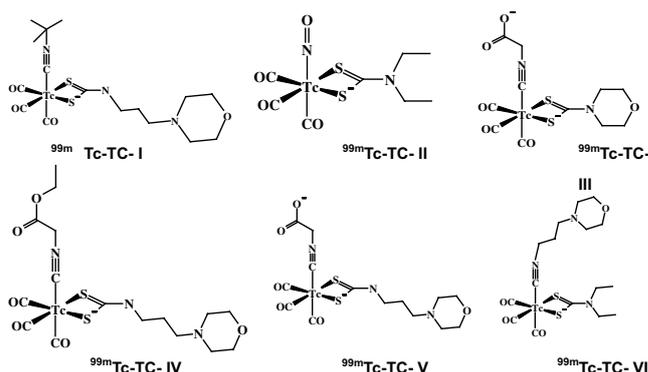


Figure 4. Structures of the ^{99m}Tc -complexes.

4. Conclusions

1. Technetium-99m in the form of pertechnetate-ions does not bind with all cells.
2. Affinity of the ^{99m}Tc -complexes to melanoma B 16 cells decreases in the following order:
 $^{99m}\text{Tc-TC-VI} >> ^{99m}\text{Tc-TC-I} >> ^{99m}\text{Tc-TC-V} > ^{99m}\text{Tc-TC-IV} > ^{99m}\text{Tc-TC-Cit}$
3. Affinity of the ^{99m}Tc -complexes to breast adenocarcinoma MCF 7 cells decreases in the following order:
 $^{99m}\text{Tc-TC-VI} > ^{99m}\text{Tc-TC-I} >> ^{99m}\text{Tc-TC-V} > ^{99m}\text{Tc-TC-II} > ^{99m}\text{Tc-TC-Cit}$
4. The cell efflux does not exceed 30% after 1.5 h re-incubation for $^{99m}\text{Tc-TC-Cit}$ and $^{99m}\text{Tc-TC-VI}$ in both cell lines.

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