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FACTORS AFFECTING LABELLING YIELD OF ^{111}In-DTPA-BSA

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Abstract

Radiolabelling of antibodies depends on a number of factors including the chemical characteristics of the nuclide and the techniques employed for its incorporation into protein. For preliminary research we used model system and investigate the influence of different factors affecting labelling. Obtained results were successfully used for further radiolabelling of antibodies with different trivalent metals.

Introduction

Development of the new radiopharmaceuticals for diagnostic and therapy purpose in nuclear medicine is still in the focus of the main research cancer centers. Antibodies labelled with alpha and beta emitters are radiopharmaceuticals with good characteristics for targeted cancer cell therapy with the minimum side effect on the surrounding healthy tissue. The purpose of this experiment was to optimize the conditions of labelling of polyclonal and monoclonal antibodies with ^{201}Tl (gamma emitter with favorable characteristics for radioimmunoscintigraphy), ^{153}Sm (beta emitter for radioimmunotherapy of solid tumors) and ^{149}Tb (for radioimmunotherapy of hematological malignancies and diffuse types of cancer) and obtain *in vitro* and *in vivo* stable radioimmunoconjugates. However, as applicability of radionuclides (^{149}Tb and ^{153}Sm) used for the labelling of antibodies in these studies, has not been investigated enough and due to the low availability and high price of these radionuclides, it was not possible to use them for preliminary experiments where all conditions of labelling of antibodies with radionuclides should be settled. As a model for these trivalent radiometals, ^{111}In was used. ^{111}In is also trivalent radiometal, gamma emitter with good radiophysical characteristics for research and easily available. Bovine serum albumin (BSA) was used as a model for Rituximab antibodies for all chemical experiments.

In the preliminary experiment for chelating of BSA with bicyclic anhydride of DTPA (cDTPA) modified method of Hnatowitch was used [1]. This indirect method includes covalent coupling of bifunctional chelating agent to the BSA (antibody) which binds the radiometal. However as the stability of obtained complex ^{111}In-DTPA-BSA was low, it was necessary to optimize the conditions of formation of the complex.

Experimental

We studied a number of factors that are influencing the labeling procedure, in order to find one which lead to high yield of labeling:

- **Solvent solution for cDTPA**: Chelating of BSA with cDTPA needs specific conditions, because anhydride cDTPA hydrolyze very fast, therefore for low concentration of protein the yield of complex DTPA-protein is low. Two solvents for dissolving of cDTPA were investigated as well as addition of the solid cDTPA to the buffer (0.1 M sodium acetate buffer pH 5.6) with BSA;
- **Buffer volume for labelling**: Radiolabelling yield was determined for two (0.1 M sodium acetate) buffer volumes 0.95mL and 0.05 mL with pH 5.6;
- **Molar ratios BSA:cDTPA** from 1:1 to 1:100 were investigated;
- **Concentration of BSA**: Two different concentrations of the BSA, 1.4mg/mL and 4.6 mg/mL were used for the preparation of the conjugate, in order to see if BSA concentration affects labeling;
- **Temperature and incubation time** of chelating: The labeling was studied for the incubation time periods from 0-60 min for two temperatures, 4 °C and 24 °C. In all time points we used the same concentrations of BSA and cDTPA (C BSA=4.6mg/mL, BSA:cDTPA=1:100).

The labeling efficiency of prepared complex DTPA-BSA with ^{111}In was followed by chromatographic methods and gel filtration on PD-10 column.

Results and Discussion

The best labelling yield (89.8%) was obtained for the molar ratio BSA: cDTPA=1:100 with cDTPA added to the buffer solution in a solid form, therefore the hydrolysis of cDTPA was prevented.

Table 1. The effect of solvent and molar ratio BSA:cDTPA on the labelling yield of ^{111}In-DTPA-BSA (%)

Buffer volume (mL)	Solvent for cDTPA	Labelling yield ^{111}InDTPA-BSA (%)		
		Molar ratio BSA : cDTPA		
		1 : 1	1 : 10	1 : 100
0.95	No solvent	/	87.7	89.8
	Chloroform	72.3	78.1	81.2
	DMSO	43.3	55.6	61.7
0.05	Chloroform	53.5	57.1	62.4
	DMSO	27.5	39.7	42.5

For the low concentration of BSA (1.4mg/mL) conjugation process can't compete with the process of hydrolysis of cDTPA and radiolabelling yield is low (42.1%). In reaction with more molecules of BSA in reaction solution (4.6mg/mL), conjugation of DTPA is facilitated, therefore more binding places for radionuclide are available and radiolabelling yield is higher (89.8%).

After 5 min. of incubation of BSA with cDTPA at room temperature labelling yield was 85%, while maximum was already obtained after 10 min. of chelating, and remained practically the same up to 24 h later.

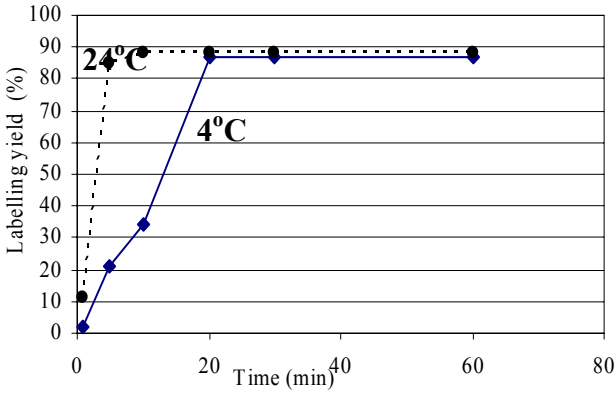

Fig. 1. The effect of incubation time and temperature on labelling yield of ^{111}In-DTPA-BSA (%)

Conclusion

These conditions (where the best results of radiolabelling yield were obtained) with minimal corrections were successfully used for labelling of different antibodies, including Rituximab antibodies, with ^{153}Sm, ^{149}Tb and later on with trivalent ^{201}Tl [2]. The citotoxicity effect of obtained radiopharmaceuticals was determined by *in vitro* cell studies and in *vivo* on healthy mice [3, 4].

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