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^{99M}TC-HEXAKIS-(2-METOXY-ISOBUTYL-ISONITRILE) (^{99M}TC-MIBI) A NEW MYOCARDIAL IMAGING AGENT: SYNTHESIS OF MIBI, OPTIMISING CONDITIONS FOR RADIOLABELLING WITH ^{99M}TC AT HIGH RADIOCHEMICAL PURITY AND *IN VIVO* BEHAVIOR

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Abstract

^{99m}Tc-MIBI is a promising radiopharmaceutical for myocardial perfusion imaging agent, but it has also shown good results in identifying several types of tumors, such as breast, lung and thyroid cancers. It is a lipophilic, cationic technetium (1) complex. In this paper a complete study on the synthesis of 2-metohy-isobutyl-isonitrile (MIBI) as well as a formulation of a lyophilized kit for labeling with ^{99m}Tc is presented. Investigation on effective factors as well as finding out the optimum parameters to obtain the highest labelling efficiency and radiochemical purity of ^{99m}Tc-MIBI complex were performed. The radiochemical purity of the labelled preparation was high (>95%). Biodistribution study performed in health male Wistar rats showed satisfactory biokinetics results. ^{99m}Tc-MIBI was accumulated in sufficient amount into the hearth tissue for myocardial perfusion imaging. MIBI in kit formulation was found to be stable and also safe for administration.

Introduction

[Hexakis-(2-metoxy-isobutyl) isonitriletechnetium (I)]$^+$, developed as potential myocardial imaging agent, is a member of the class of lipophilic hexakisisonitriletechnetium (I) cationic complexes, [Tc(CNR)]$^+$, where R is the alkyl group such as methyl, ethyl, n-propyl, tertiary butyl or metoxy isobutyl subtituents [1]. It this +1 complex, Tc in the center is symmetrically bounded to six monodentate CNR ligands resulting in stable cationic complexes. Isonitriles stabilize Tc(I) by their p-acceptor properties. These compounds don't undergo the *in vivo* reductive mechanisms in the human heart owing to the low oxidation state of technetium. An investigation into the mechanism of uptake has led to the belief that cations such as [Tc(MIBI)$_6$]$^+$ accumulate *via* a diffusion mechanism and electrostatic binding due to a high mitochondrial membrane potential. The lipophilicity of the complex is known to be important for uptake into the heart. Tc(I) oxidation state is accessible directly from pertechnetate (^{99m}TcO$_4^-$) as the complex is obtained by the reaction of ^{99m}TcO$_4^-$ and tetra-(2-metoxy-isobutyl-isonitril) copper tetrafluoroborate [Cu(MIBI)$_4$] BF$_4$ and in presence of SnCl$_2$ as reducing agent [2, 3].

Material and Methods

The synthesis of MIBI and chelate $[Cu(MIBI)_4]$ BF_4 was based on a method previously reported [4], with certain modifications. The compounds were characterized by elemental analysis, IR and ^{1}H-NMR spectroscopy. After optimization of reaction parameters, MIBI was prepared as a freeze dried kit. The labelling was performed by adding of 1-5 ml of $^{99m}TcO_4^-$ ($^{98}Mo/^{99m}Tc$-generator, Vinča) and heating for fiveten minutes in boilling wather bath. Analysis of the product included the radiochemical quality control, determination of pharmacokinetical parameters as well as biodistribution study. Radiochemical purity was assayed by Whatman 3MM paper chromatography and ITLC-SG in ethylacetate and two solvent system (acetone/saline) respectively. The standard trichloracetic acid (TCA) precipitation method was used for determining the binding of ^{99m}Tc-MIBI to protein. All lipophilicity measurements were done by solvent extraction method with n-octanol. The biodistibution studies were carried out using health male Wistar rats (n=4-6). Animals were sacrified at different times and samples of tissues or organs of interest were removed for assay of radioactivity.

Results and Discusion

The different concentrations and molar ratios of $[Cu(MIBI)_4]BF_4$ and $SnCl_2{\times}2H_2O$ as well as the volume of $^{99m}TcO_4^-$ elute used for labeling, had a great influence on the labelling yield and stability of the preparation (Table 1 and 2). The optimum quantity of Sn(II) added into kit formulation was investigated by preparing MIBI kits containing various concentrations of Sn(II), while amounts of MIBI and others components were kept constant. The experimental results confirmed that concentration ratio: [Sn]/[MIBI]=1/13.5 and the presence of 0.025 mg/ml of $SnCl_2{\times}2H_2O$ in freeze-dried kit, gave satisfactory biological behavior in rats.

Table 1. The effect of Sn(II) concentration on radiochemical purity results of ^{99m}Tc-MIBI (Whatman 3MM, Ethyl Acetate), V_{TcO4-}=3 ml

Time after labelling	30 (min)		300 (min)	
[Sn(II)] mg/ml	TcO_2+ TcO_4^- Rf=0.0	Tc-MIBI Rf=0.6	TcO_2+ TcO_4^- Rf=0.0	Tc-MIBI Rf=0.6
0.25	4.5±0.2	95.5±0.2	5.1±0.5	94.9±0.5
0.025	2.2±0.2	98.0±0.9	2.4±0.4	97.6±0.8

Table 2. The effect of $^{99m}TcO_4^-$ volume on radiochemical purity results of ^{99m}Tc-MIBI

V_{TcO4-} (ml)	Labelling Yield (%)	
	TcO_2+ TcO_4^-	Tc-MIBI
1	9.4±0.7	90.6±0.9
2	6.1±0.3	93.9±0.8
3	2.2±0.6	97.8±0.6
4	2.8±0.8	97.2±0.8
5	3.3±0.9	96.7±0.3

Reconstitution of the kit with 1-5 ml of $^{99m}TcO_4^-$ showed high radiochemical purity and stability. The freeze dried kit for ^{99m}Tc-MIBI is stable at least 5 h after labelling. The effect of heating on the radiochemical yield was tested. ^{99m}Tc-MIBI was pre-

pared with and without heating. Without heating much lower radiochemical purity results were obtained. The percentage of ^{99m}Tc-MIBI bound to protein was very high (94.8%). N-octanol-buffer distribution pH profiles showed that the distribution coefficient was around 0.5 and independent on pH. From values of radioactivity measured per organ of interest, the percentages of radioactivity related to administrated dose were determined. *In vitro* studies showed the significant hearth uptake and low blood and liver uptake (Table 3). The effect of Sn(II) concentration on *in vivo* behavior was remarkable.

Table 3. Effect of [Sn (II)] on biodistribution studies results of ^{99m}Tc-MIBI on Wistar rats, % Administered dose ± SD per g (%/g), ($V_{99mTcO4}$=5 ml)

Organ	[Sn(II)]= 0.025 mg/ml	[Sn(II)]= 0.25 mg/ml
Heart	3.12±0.38	3.77±0.21
Blood	0.02±0.01	0.03±0.01
Lungs	0.33±0.05	0.67±0.10
Liver	0.84±0.01	1.03±0.31
Spleen	0.46 ±0.12	0.32±0.12
Kidneys	2.17±0.30	1.75±0.20
Stomach	0.73±0.01	0.45±0.17
Intestine	2.20±0.12	1.12±0.18
Heart/Blood	198.89	132.59
Heart/Lungs	9.414	5.65
Heart/Liver	3.71	3.67

Conclusion

MIBI was prepared as freeze dried form. Reconstitution of the kit performed with 2-5 ml of $^{99m}TcO_4^-$ showed high radiochemical purity. A satisfactory biodistribution of ^{99m}Tc-MIBI behaviour in helthy test animals was obtained. Aditional clinical studies could confirm the promising characteristic of ^{99m}Tc-MIBI.

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