Preparation of $^{186}$Re-MIBI Complex for Myocardial Perfusion Imaging as Potential Replacement of Analogues $^{99m}$Tc-MIBI

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ABSTRACT

The aim of the present study is to label 2-methoxyisobutyl-isonitrile (MIBI) compound with pure $^{186}$Re and study the optimum conditions to prepare the $^{186}$Re-MIBI complex as a stable contrast agent for myocardial perfusion imaging. From the obtained data the complexation of MIBI with $^{186}$Re was carried out using 1mg MIBI, 1mg SnCl$_2$, 3 mg gentesic acid and 1 ml $^{186}$Re(37MBq) at pH 2 in a boiling water bath for 30 min. The biodistribution studies in mice indicate that, the complex was cleared from the body by kidneys to urinary bladder and finally was eliminated from the body by urine. $^{186}$Re-MIBI demonstrated satisfactory heart uptake and retention like $^{99m}$Tc- MIBI (8.94% dose/organ at 5 minutes), blood clearance was fast, while liver activity deceased by time and negligible activity in the lungs. The obtained data showed that $^{186}$Re-MIBI as a potential replacement of $^{99m}$Tc-MIBI for myocardial perfusion imaging.

Key Words: $^{186}$Re/ $^{186}$Re-MIBI/ Radiochemical Yield/ Biological Distribution/ Myocardial Perfusion Imaging.

INTRODUCTION

The development of technetium complexes as potential radiopharmaceuticals is facilitated by use of rhenium of the group VII B congener of technetium. Rhenium generally produces complexes with similar physical and biodistribution properties to those of technetium (1). $^{186}$Re showed to be one of the most promising candidates. $^{186}$Re is characterized by reasonable half life of 91 hours, $\beta^-$ emission and essentially single $\gamma$-emission of 137 keV very similar to $^{99m}$Tc which induced ideal for imaging (2,3). $^{188}$Re is produced in the reactor by direct irradiation of $^{185}$Re in a (n, $\gamma$) reaction. One of the most widely radionuclide used in Single Photon Emission Computed Tomography (SPECT) imaging agents in routine nuclear medicine, $^{99m}$Tc-sestamibi (Cardiolite) as shown in Fig. (1), is a calassical organometallic compound originally developed as a myocardial perfusion agent (4). Some attempts were done to prepare the terminal Re≡N bond using oxalate ions in presence of stannous chloride (5). Also $^{188}$Re-sestamibi complex was prepared with low radio labeling efficiency (6).

Fig. (1): The structure of $^{99m}$Tc-MIBI.
In our study to prepare $^{186}$Re-MIBI as $^{99m}$Tc-MIBI analogues using gentesic acid as an antioxidant is carried out (7). The aim was to find a method to radiolabel the MIBI with $^{186}$Re in a stable fashion.

**EXPERIMENTAL**

**Materials and Methods:**

Rhenium oxide $\text{Re}_2\text{O}_7$ (99.9% pure) was purchased from Aldrich Chemical Company. Stannous chloride, gentesic acid and potassium perrhenate were purchased from Sigma Chemical Company. MIBI used in this study is a gift from National Center for Scientific Research Athens, Greece (DEMO).

**Production of $^{186}$Re:**

It was produced by irradiation of 0.1g of $^{185}$Re in the 22MW Egyptian Research Reactor (ETRR-2) by thermal neutron flux of $10 \times 10^{14}/\text{cm}^2\cdot\text{s}$ for 5h and cooling time 6 days (7). The irradiated target was dissolved in 2-3 ml of distilled water. The activity was measured by Isotope Dose Calibrator and found to be $\approx 35\text{mCi} \ 186\text{Re}$.

**Radiolabeling:**

Experiments were carried out at different concentrations of MIBI (0.5-4mg) and SnCl$_2$ (0.1-2mg), the pH was studied from 1 to 5 in presence of gentesic acid (1-5mg). The reaction mixture was carried out at room temperature (25±1°C) up to 100°C at different reaction times (10-60min), also the effect of carrier potassium perrhenate (KReO$_4$) was investigated from 0 to 0.5mg.

**Determination of the Radiochemical Yield:**

Radiochromatographic methods were used for the determination of the radiochemical yield.

**Thin layer chromatography (TLC):**

TLC was performed as follows: 5µl of the reaction mixture was spotted at 2cm from the lower end of the TLC-Silica Gel sheet. The strip was developed in an acetone or saline until the solvent moved to 10cm. The strip was cut into segments of 1cm each and the radioactivity was measured.

**Electrophoresis:**

Electrophoresis was done using EC 3000P-series 90 programmable power and chamber supply units using cellulose acetate strips (45cm). These strips were pre-treated with 0.02 M phosphate buffer pH 7.5. 5µl of the reaction mixture was spotted at 5cm from the lower end of the strip. The electrophoretic paper was run out for 1h at 300V .The strip was dried and cut into segments of 1cm each and the radioactivity was counted.

**In-vitro Stability of $^{186}$Re-MIBI:**

The in-vitro stability of the complex was investigated as a function of time from 1h to 24h.

**Biodistribution study:**

Organs distribution study were carried out in a groups of three male Albino mice weighing 28-30g, each animal was injected in tail vein with 0.2 ml containing 7-10MBq of $^{186}$Re-MIBI complex.
mice were anaesthetized by ether at 5, 30 and 60 min post injection (PI). Samples of tissues and organs were removed, weighed and their radioactivity content was measured in a gamma counter.

RESULTS AND DISCUSSION

Production of $^{186}$Re:

Specific activity ranged from 16 to 18 GBq/mg was obtained. The radionuclide purity was determined by gamma spectroscopy by counting an aliquot of the obtained solution on a High Pure Germanium (HPGE) detector. Gamma spectrum of Re is shown in Fig. (2). The spectrum obtained shows the characteristic peak at 137 keV related to $^{186}$Re.

![Gamma Spectrum of $^{186}$Re](image)

Preparation of $^{186}$Re-MIBI:

$^{186}$Re-MIBI complex can be prepared using 1 mg of MIBI, 1 mg of stannous chloride, 3 mg gentesic acid and 1 ml of $^{186}$ReO$_4^-$ (37 MBq) was added after adjusting pH to 2. The reaction mixture solution purged with nitrogen and heated in a boiling water bath for 30 min reaction time.

Radiochemical studies:

MIBI coordinates in a hexakis arrangement to rhenium(I) is the same as $^{99m}$Tc-MIBI, like other lipophilic technetium isonitrile cations (8,9). MIBI was successfully labeled with $^{186}$Re with radiochemical yield >95%. The radiochemical analysis was achieved by TLC-SG sheets using acetone as a solvent (Table 1). The complex remained at the point of spotting $R_f$ of approximately 0.2 and the free $^{186}$ReO$_4^-$ moved toward solvent front (0.9-1.0), using TLC/saline (Table 1). $^{186}$Re-MIBI moved with solvent front $R_f$ = 0.65 and reduced hydrolysed $^{186}$ReO$_2$ at 0.0, if present, would be expected to remain at the point of spotting. The obtained results were in a complete agreement with Das et al. (10).
Table (1): Radiochromatography of $^{186}$Re-MIBI.

<table>
<thead>
<tr>
<th>Support</th>
<th>Solvent</th>
<th>Species</th>
<th>$R_f$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLC</td>
<td>Acetone</td>
<td>$^{186}$ReO$_4$</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$^{186}$Re-MIBI</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>$^{186}$Re-RH</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$^{186}$Re-MIBI</td>
<td>0.65</td>
</tr>
</tbody>
</table>

Electrophoresis was performed under the same conditions stated before. Free perrhenate and $^{186}$Re-MIBI moved from the point of spotting toward the cathode as shown in Figure (3). The free $^{186}$ReO$_4$ migrates towards the anode but the reduced hydrolyzed rhenium remains at the point of spotting.

![Fig. (3): The electrophoresis analysis of $^{186}$Re-MIBI.](image)

Factors Affecting the Radiochemical Yield of $^{186}$Re-MIBI:

Effect of MIBI amount:

The effect of the amount of MIBI on the radiochemical yield of $^{186}$Re-MIBI complex was studied at pH 2. In Fig. (4) the labeling yield was found to be more than 95% when the amount of MIBI was 1mg. At low quantities of MIBI 0.5mg, the radiochemical yield was 85%, this due to incomplete formation of $^{186}$Re-MIBI complex. No increasing of the radiochemical yield was obtained by increasing the amount of MIBI than 1mg. The results obtained for MIBI are in a complete agreement with the data reported by Verdera et al. (11).

Effect of stannous chloride content:

It is necessary for labeling with rhenium to reduce Re$^{7+}$ to its more reactive lower oxidation state as reported by Griffiths et al. (12), similar to $^{99m}$TcO$_4^-$, the reduction of $^{186}$ReO$_4^-$ is generally accomplished by the addition of anhydrous SnCl$_2$ in acidic medium. However, due to the fact that Re is more difficult to reduce than Tc-analogue, a considerably higher concentration of SnCl$_2$ is required for radiolabeling $^{186}$Re (13). The effect of SnCl$_2$ content on the radiochemical yield was studied in the range of 0.2-2 mg (Fig. 5). At low concentration 0.2 mg the labeling yield was about 65%, this due to...
insufficiency of stannous chloride to reduce perrhenate. In Fig. (5) the data show that more excess of stannous chloride at 1mg high labeling yield (>95%) (14-16) was achieved. At 2mg of stannous chloride no change in the radiochemical yield was observed.

Fig. (5): Effect of SnCl₂ content on the radiochemical yield of ¹⁸⁶Re-MIBI.

Effect of pH:

Effect of the pH on the radiochemical yield was studied in pH range 1-5. Fig. (6) shows that highest radiochemical yield more than 95% at pH 2, this due to higher degree of the reduction of perrhenate (17) and the acidity of reaction mixture also played an important role in complex formation (18). At pH more than 2 the radiochemical yield was decreased, this due to the stability of reduced rhenium decreased with increasing pH from 3 to 5 (17).
Influence of antioxidant content:

The addition of antioxidant gentesic acid is known from literature\(^{(19-21)}\) as the way how to enhance the reducing environment to prevent a reoxidation of reduced rhenium to perrhenate. The influence of gentesic acid content on the radiochemical yield was investigated in the range 1-5 mg Fig. (7). The addition of gentesic acid causes the increase of reduction yield > 90\(^{(17)}\). The radiochemical yield increased by increasing gentesic acid content up to more than 95% was obtained at 3 mg gentesic acid. After that no further increase in the labeling yield with increasing the gentesic acid content up to 5 mg, in comparison with 58% reduction yield without gentesic acid\(^{(17)}\).

![Fig. (6): Effect of pH on the radiochemical yield of \(^{186}\)Re-MIBI.](image1)

![Fig. (7): Effect of gentesic acid content on the radiochemical yield of \(^{186}\)Re-MIBI.](image2)

Effect of reaction time:

The influence of reaction time on the radiochemical yield of \(^{186}\)Re-MIBI complex was investigated (Fig 8). A gradual increase of the results indicates that highest labeling yield (95.5%) was achieved at 30 min. This is in a good agreement with the data obtained by Das et al.\(^{(10)}\). Hence, 30 min would be enough to complete formation of \(^{186}\)Re-MIBI. No more increase in the labeling yield more than 30 min was achieved.
Fig. (8): Effect of reaction time on the radiochemical yield of $^{186}\text{Re-MIBI}$.

**Effect of reaction temperature:**

The influence of reaction temperature on the radiochemical yield of $^{186}\text{Re-MIBI}$ (Fig. 9). It was found that high radiochemical yield more than 95% at 100°C $^{(22)}$. At room temperature rhenium could not be easy to reduce.

Fig. (9): Effect of reaction temperature on the radiochemical yield $^{186}\text{Re-MIBI}$.

**Effect of carrier content:**

The influence of adding carrier KReO$_4$ on the percent of radiochemical yield of $^{186}\text{Re-MIBI}$ complex was investigated in the range 0-0.5mg. Table (2) shows that the labeling yield is not affected by the presence of KReO$_4$ carrier and about 95.5% labeling yield was achieved with and without carrier as reported by Hashimoto $^{(7)}$.

<table>
<thead>
<tr>
<th>KReO$_4$ Carrier, mg</th>
<th>Radiochemical yield, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>95.5±0.4</td>
</tr>
<tr>
<td>0.1</td>
<td>95.5±0.6</td>
</tr>
<tr>
<td>0.2</td>
<td>95.4±0.4</td>
</tr>
<tr>
<td>0.3</td>
<td>95.3±0.3</td>
</tr>
<tr>
<td>0.4</td>
<td>95.6±0.5</td>
</tr>
<tr>
<td>0.5</td>
<td>95.6±0.1</td>
</tr>
</tbody>
</table>
In-vitro stability of $^{186}$Re-MIBI:

The in-vitro stability of the complex shows that the complex is stable from 1 to 6h (Table 3).

Table (3): Stability of $^{186}$Re-MIBI complex at room temperature.

<table>
<thead>
<tr>
<th>Time, h</th>
<th>Radiochemical yield, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>95.5±0.8</td>
</tr>
<tr>
<td>2</td>
<td>94.8±0.3</td>
</tr>
<tr>
<td>4</td>
<td>95.2±0.6</td>
</tr>
<tr>
<td>5</td>
<td>95.0±0.2</td>
</tr>
<tr>
<td>6</td>
<td>94.5±0.5</td>
</tr>
</tbody>
</table>

Biological Distribution:

Biodistribution studies in mice indicate that $^{186}$Re-MIBI complex exhibits a tissue distribution similar to $^{99m}$Tc-MIBI with rapid extraction from the blood into perfused myocardium, liver, kidneys and muscles (Table 4). At 5 min PI, the heart/lung ratio per gram basis is greater than one for $^{186}$Re-MIBI indicating potential for early visualization of the heart as reported by Plossl et al. (23) as shows in (Table 4). Studies were performed at 5, 30 and 60 min PI. $^{186}$Re-MIBI demonstrated a satisfactory heart uptake and retention like $^{99m}$Tc-MIBI (8.34% dose/organ at 5 min PI and 7.42% at 60 min PI) and this values found to be more than as reported by Walther et al. (24). Blood clearance was rather fast while liver activity was decreased by time (8.34% dose/organ at 5 min to 7.42% dose/organ at 60 min PI) and lungs activity were found negligible for $^{186}$Re-MIBI (2.34 and 1.24% dose/organ at 5 and 60 min PI, respectively). The obtained data proved that $^{186}$Re-MIBI is rapidly accumulated and retained in heart tissue and also could be replaced of $^{99m}$Tc-MIBI.

Table (4): Biodistribution of $^{186}$Re-MIBI complex in mice.

<table>
<thead>
<tr>
<th>Organs and body fluids</th>
<th>% Injected dose / organ and body fluids at different time intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5min</td>
</tr>
<tr>
<td>Blood</td>
<td>0.49±0.10</td>
</tr>
<tr>
<td>Liver</td>
<td>8.34±2.00</td>
</tr>
<tr>
<td>Heart</td>
<td>8.94±0.04</td>
</tr>
<tr>
<td>Kidneys</td>
<td>20.0±0.44</td>
</tr>
<tr>
<td>Stomach</td>
<td>2.30±0.20</td>
</tr>
<tr>
<td>Intestines</td>
<td>4.30±0.30</td>
</tr>
<tr>
<td>Spleen</td>
<td>3.50±0.20</td>
</tr>
<tr>
<td>Muscles</td>
<td>1.93±0.01</td>
</tr>
<tr>
<td>Lungs</td>
<td>2.34±0.03</td>
</tr>
</tbody>
</table>

REFERENCES