Preparation and Biological Evaluation of $^{99m}$Tc-TMPP as a Novel Agent for Tumor Diagnosis

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Received: 20/12/2014 Accepted: 9/4/2015

ABSTRACT

TMPP (5, 10, 15, 20 tetrakis (4-methoxyphenyl) 21H, 23H porphyrin) was labeled with technetium-99m via direct labeling technique using stannous chloride as a reducing agent. The optimum conditions that gave high labeling yield (95.2%) of $^{99m}$Tc-TMPP complex were achieved by using 3mg TMPP, 100μg SnCl$_2$.2H$_2$O, at pH 3 and 30min reaction time. Accumulation of $^{99m}$Tc-TMPP complex was studied in tumor bearing mice. After intravenous injection of $^{99m}$Tc-TMPP complex, tumor radioactivity uptake was high compared to normal muscle uptake and it was 7.76 folds after 30 min. Tumor to blood ratio was 2.36 after 30 min.

Key Words: Technetium-99m / TMPP / Labeling / Biodistribution / Hypoxia / Imaging

INTRODUCTION

Porphyrrins are essential in many biological processes of normal metabolism of living organisms, such as oxygen transport, photosynthesis, etc. because they form highly stable complexes with many metals. Hence, they have many applications in biomedical and chemical analysis (1). Also, porphyrins have characteristics of selective accumulation in tumor tissues of animals and human models (2). The ability of porphyrin derivatives to accumulate in the neoplastic cells is demonstrated in photodynamic therapy (PDT) which is a medical treatment modality employing a combination of light and photosensitizing drug to develop reactive oxygen species (ROS) in tumor cells (3-5). ROS induced by photosensitizing drugs are preferentially accumulated in tumor cells, leading to cell damage in several subcellular organelles (3,4,6). Porphyrrins are well-recognized photosensitizers drugs for the treatment of cancer (3-5). Moreover, porphyrrins as photosensitizers have the advantages of lack of toxicity in the dark, stable composition, selective photosensitization of tumor tissue and high synthesis of ROS (4,7). They are also well known to show phototoxicity and selective accumulation in tumor cells. Although PDT is clinically well exploited, this technique has several disadvantages such as masking of tumor due to hemorrhage, less effectiveness in treating of large tumors, low sensitivity of detection, etc. (8-10). These advantages of porphyrrins and disadvantages of PDT open the way to make labeling of porphyrrins derivatives with suitable radionuclides that may improve the efficacy of porphyrrins in diagnosis of tumors.

$^{99m}$Tc is a widely used radionuclide in radioactive tracer investigations as single-photon emission computed tomography (SPECT) imaging agent owing to its appropriate half-life (about 6 hours) that ensures that the patient is not exposed to unnecessary radiation. $^{99m}$Tc is also of favorable energy (140 KeV) of $\gamma$-ray yielding a high counting efficacy (11-18).

This work aims at labeling TMPP with $^{99m}$Tc under different experimental conditions and investigation of the potential use of $^{99m}$Tc-TMPP (prepared under the optimum conditions) as a tumor imaging agent using a mouse model.
EXPERIMENTAL

Chemicals:

TMPP was purchased from Aldrich Chemical Company. $^{99m}$Tc generator was purchased from (Elutic, Brussels, Belgium). All other chemicals were purchased from Merck and they were of reactive grade.

METHODS

Method of Labeling:

Three milligrams of TMPP were transferred to a 10ml vial then the vial was evacuated. A solution containing 100µg SnCl$_2$·2H$_2$O was added and the pH of the reaction mixture was adjusted to 4 then the volume of the mixture was completed to one ml by N$_2$ purged distilled water. One ml of freshly eluted $^{99m}$TcO$_4^-$ (400MBq) was added to the above mixture. The reaction mixture was well shaken and allowed to react at room temperature for sufficient time (30min) required to complete the reaction.

Analysis:

The percent labeling yield of the labeled $^{99m}$Tc-TMPP complex was determined by using paper and HPLC chromatographic techniques.

Paper Chromatographic Technique:

The percent labeling yield was determined by using the ascending paper chromatographic technique. Strips of Whatman No.3 paper chromatography, 10 cm length and 1.5 cm width, were marked gently with a pencil at a distance of 2 cm from the lower end lined into sections 0.5 cm each up to 7 cm. A spot from the reaction mixture was applied using a hypodermic syringe and then the strip was developed in an ascending manner in a closed jar filled with N$_2$ gas to prevent oxidative decomposition of the labeled $^{99m}$Tc-TMPP spot. The developing solvents namely; acetone and saline were purged with N$_2$ gas for the same purpose. After complete development, the strips were dried and cut into fragments of 0.5 cm each. Then the sections were counted in a well-type $\gamma$-scintillation counter. The organic solvent acetone was used to calculate the percent of free $^{99m}$TcO$_4^-$ and saline was used to calculate the amount of reduced hydrolyzed technetium-$^{99m}$ (colloid).

HPLC Chromatographic Technique:

Before the HPLC analysis of the labeled compound, a cold solution of TMPP was injected to the column (RP18 – 250×4 mm, 5µm, Lischrosorb) build in HPLC Shimadzu model consisting of pumps LC-9A with a Rheohydroin injector and UV spectrophotometer detector (SPD-6A) adjusted to the wave length 254 nm. The column was eluted with the isocratic solvent methanol: water 70:30 and the flow rate was adjusted to 1 ml / min. TMPP gives a peak at R$_t$ 4.3 min. Then, 10 µl of the reaction mixture containing $^{99m}$Tc-TMPP was injected to the column of HPLC under the same conditions mentioned before and the fractions of 1 ml were collected and counted using 3-inch NaI (TI) well-type crystal coupled to SR-7 scaler ratemeter so that a radiochromatogram can be obtained.

Induction of Tumor in Mice:

Exactly 0.2 ml solution of Ehrlich Ascites Carcinoma was then injected intramuscularly in the right thigh of female Swiss Albino mice to produce a solid tumor. The animals were maintained till the tumor development was apparent (10-15 days). The parent tumor line (Ehrlich Ascites Carcinoma) was withdrawn from 7 days old donor female Swiss Albino mice and diluted with sterile physiological saline solution to give 12.5 x 10$^6$ cells/ml.

Biodistribution Study in Mice:

In vivo biodistribution studies were done in groups of three female Albino mice where each animal was injected in the tail vein with 0.2 ml solution containing 5-10 kBq of $^{99m}$Tc-TMPP. The
mice were put in metabolic cages for the required time. The mice were sacrificed by cervical dislocation in groups at various time intervals after injection and the organs or tissues of interest were removed, weighed and counted. Correction was made for background radiation and physical decay during the experiment.

**RESULTS AND DISCUSSION**

Radiochemical purity and stability of $^{99m}$Tc-TMPP complex were assessed by paper and HPLC chromatographic methods. In paper chromatography using acetone as the developing solvent, free $^{99m}$TcO$_4^-$ moved with the solvent front ($R_f$=1), while $^{99m}$Tc-TMPP and reduced hydrolyzed technetium remained at the point of spotting. Reduced hydrolyzed technetium was determined by using saline as the mobile phase where reduced hydrolyzed technetium remained at the origin ($R_f$ = 0) while other species migrate with the solvent front ($R_f$ =1). By subtracting the sum of the percent of colloid and free pertechnetate from 100% the radiochemical purity was calculated. The radiochemical purity is the mean value of three experiments.

**HPLC Analysis of the $^{99m}$Tc-TMPP:**

The radiochromatogram was drawn as presented in Figure (1) which shows two peaks one at retention time $R_t = 2$ min. which corresponds to the free pertechnetate, while the second peak collected at $R_t = 5.5$ min. corresponds to $^{99m}$Tc-TMPP.

**Factors Affecting the Percent Labeling Yield**

**Effect of TMPP Amount:**

Fig.2. shows that at 1mg TMPP, the labeling yield of $^{99m}$Tc-TMPP complex was 76.2% where this low labeling yield was due to the fact that the substrate concentration is insufficient to complex all reduced technetium. By increasing the amount of TMPP, the labeling yield increased and reached the maximum value of 95.2% at 3 mg TMPP. By increasing the TMPP amount over 3mg, the labeling yield slightly decreased again till reaching 66.7% at 10mg TMPP.

**Effect of SnCl$_2$.2H$_2$O Content:**

Fig.3. shows that below 100µg SnCl$_2$.2H$_2$O, the percent labeling yield was low because SnCl$_2$.2H$_2$O is not enough for complete reduction of pertechnetate to form $^{99m}$Tc-TMPP complex. This was proved by two points, at 50µg SnCl$_2$.2H$_2$O the quantity of free pertechnetate was 17.9% then
decreased to 6.6% at 75µg of SnCl₂.2H₂O. The optimum labeling yield was obtained at 100µg SnCl₂.2H₂O at which a maximum labeling yield of 95.2% was obtained. When excess SnCl₂.2H₂O content is used, >100µg, the labeling yield decreased again (77.3% at 150µg SnCl₂.2H₂O) and the main impurity reduced hydrolyzed technetium (21.1% at 150µg SnCl₂.2H₂O) because excess SnCl₂.2H₂O was converted to colloid.

Fig (2): Effect of TMPP amount on the labeling yield of ⁹⁹ᵐTc-TMPP.

Fig. (3): Effect of SnCl₂.2H₂O on the percent labeling yield of ⁹⁹ᵐTc-TMPP.
Effect of pH of the Reaction Medium:

Figure 4 shows that, the labeling yield of $^{99m}$Tc-TMPP is dependent on the pH of the reaction mixture in the range from 2 to 6. At pH 2, the difference between free pertechnetate and $^{99m}$Tc-RH was not so high, the free $^{99m}$TcO$_4^-$ and $^{99m}$Tc-RH were equal to (2.8 and 6.7%, respectively). The optimum labeling yield of 95.2% was obtained at pH 3. Above pH 3, the yield decreased again due to colloid formation. At pH 6, $^{99m}$Tc-RH was the main impurity and was equal to 29.6%.

![Figure 4: Effect of pH of the reaction mixture on the labeling yield of $^{99m}$Tc-TMPP.](image)

**Effect of Reaction Time and In-Vitro Stability:**

As shown in Fig 5, labeling of TMPP with $^{99m}$Tc was carried out at room temperature for different time intervals at the optimum conditions which is (3mg TMPP , 100µg SnCl$_2$.2H$_2$O and pH3), the reaction is completed at 30 min reaching labeling yield 95.2% which slightly decreased after 60min to 86.2% and then remains stable at 85% for 8 hours.

![Figure 5: Effect of time on the labeling yield of $^{99m}$Tc-TMPP complex.](image)
Biodistribution of \(^{99m}\text{Tc}\)-TMPP Complex:

*In-vivo* biodistribution studies of \(^{99m}\text{Tc}\)-TMPP in solid tumor bearing mice was found to be greatest in blood, heart and stomach (18.97, 11.49 and 2.72%, respectively) at 15 min post injection and lowest in left normal muscle and bone (2.93 and 6.95%, respectively) (Table 1). The biodistribution of \(^{99m}\text{Tc}\)-TMPP in the right thigh (inoculated) was greater than that of the left one. The uptake of \(^{99m}\text{Tc}\)-TMPP in right thigh significantly increased with time and was equal to 15.99 and 14.7% per gram at 1 and 4h, respectively, indicating that \(^{99m}\text{Tc}\)-TMPP delivers \(^{99m}\text{Tc}\) to the tumor sites with a sufficient percentage for imaging.

**Table (1):** Biodistribution of \(^{99m}\text{Tc}\)-TMPP complex in Albino mice bearing EAC.

<table>
<thead>
<tr>
<th>Organs &amp; body Fluids</th>
<th>% Injected dose / gram organs and body fluids at different times post injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 min</td>
</tr>
<tr>
<td>Blood</td>
<td>18.97±0.94</td>
</tr>
<tr>
<td>Kidneys</td>
<td>6.32±0.316</td>
</tr>
<tr>
<td>Liver</td>
<td>2.98±0.14</td>
</tr>
<tr>
<td>Spleen</td>
<td>6.3±0.315</td>
</tr>
<tr>
<td>Intestine</td>
<td>8.45±0.42</td>
</tr>
<tr>
<td>Stomach</td>
<td>2.72±0.13</td>
</tr>
<tr>
<td>Lungs</td>
<td>4.2±0.21</td>
</tr>
<tr>
<td>Heart</td>
<td>11.49±0.57</td>
</tr>
<tr>
<td>Normal muscle</td>
<td>2.93±0.14</td>
</tr>
<tr>
<td>Solid tumor muscle</td>
<td>9.33±0.46</td>
</tr>
<tr>
<td>Bone</td>
<td>6.95±0.34</td>
</tr>
</tbody>
</table>

Reaction conditions: 3mg TMPP, 100µg SnCl\(_2\).2H\(_2\)O, pH 3 and 30 min reaction time, n=3

**CONCLUSION**

\(^{99m}\text{Tc}\)-TMPP was prepared via direct labeling technique and a high labeling yield of 95.2% was obtained using (3mg TMPP, 100µg SnCl\(_2\).2H\(_2\)O, pH 3 and 30 min reaction time) then biodistribution is performed.

This study demonstrates a specific accumulation of \(^{99m}\text{Tc}\)-TMPP into hypoxic tumor *in-vivo*.

**REFERENCES**

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