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Radiolabelling and Biological Assessment of ^{99m}Tc-Mebeverine as a Possible Tracer for Solid Tumor Diagnosis

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NUCLEAR medicine is one the most important fields to inspect and treat tumors by radiation or by introducing labeled radiopharmaceuticals, which are in the spot of our interest. In this study, the authors investigated the possibility of using mebeverine as a solid tumor marker. Mebeverine was labeled with Technetium (^{99m}Tc-Meb) reaching a radiochemical yield (RCY) of 97% via a reduction reaction using $SnCl_2$. The tracer showed a high stability in the serum for 12 hours which is essential to avoid any undesired accumulations in the non-target organs. Its bioevaluation in normal and induced tumored mice was performed to identify the capability of the use of the tracer for the diagnosis of solid tumors. The uptake by the tumor was 14.2% after 30 min of injection and increased to 18.5%, viewing a decline after 3 hours, this could be attributed to that washout to the tumored muscle extravasation. Tumored /Non-Tumored (T/NT) ratio was applied, the ratio reached 3.14 which shows the selectivity of ^{99m}Tc-Meb to the solid tumor induced in the right thigh.

Keywords: Mebeverine, Serum stability, Solid tumor, ^{99m}Tc, T/NT ratio, Tumored mice.

Introduction

Inflammatory bowel diseases (IBS), infectious diseases, and colon cancer are diseases causing colon disorders (Krishnaiah & Satyanarayana, 2001). Irritable bowel syndrome is a provocative bowel disease (Defrees & Bailey, 2017) that is influenced by many aspects such as microbiota, probiotic, gut-brain-axis, and treatment modality (Raskov et al., 2016). Antispasmodics drugs are used for the treatment of IBS, without substantial adverse effects. There was a study that Simethicone enhances the properties of the antispasmodic agents (Martínez-Vázquez et al., 2012). Mebeverine is an antispasmodic drug (Darvish-Damavandi et al., 2010) that manages IBS and controls bowel function (Talley, 2001). Mebeverine is found in two enantiomeric forms, which have variant pharmacokinetic outlines (Hatami et al., 2012). Mebeverine can eradicate signs related to post-cholecystectomy gastrointestinal spasms (Maev et al., 2018). The drug has a direct effect on the smooth muscle of the GIT (Abdelhady et al., 2003; Hosney et al., 1996). Mebeverine-loaded ethosomal vesicles are considered a promising delivery vehicle for local anesthetic effect (Hasan et al., 2020).

Radiotracers are synthetic combinations, by which one or more atoms were replaced by a radionuclide. Radioisotopes of technetium and iodine have been applied broadly to track the path of the biochemical reactions (Rennie, 1999). In the present study, Mebeverine presented as in Fig. 1 was labeled with ^{99m}Tc ,and an *in-vivo* tissue distribution study of ^{99m}Tc-Mebeverine, in normal and solid tumor-bearing male Swiss albino mice were explored.

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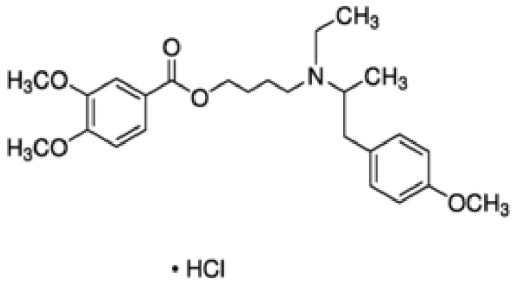

Fig. 1. Mebeverine

Materials and Methods

Materials

All materials were of an analytical grade. Mebeverine was kindly obtained from EIPICO Company, Egypt.

Equipment

The gamma counter (Nucleus Model 2010) was performed to the extent of the radioactivity using a well-type NaI (Tl) crystal.

HPLC (Merck-Hitachi Model), containing L-6000 pump and an L-4000 UV Spectrophotometric, stationary phase comprising a reversed phase C18 column (250mm x 4.5mm, 5 μm).

Animals

Male albino mice weighed 30-35g were used, they were obtained from the Animal House of Nile Pharmaceutical Company, Cairo, Egypt. The animals were under controlled conditions by introducing typical food pellets and hygienic water.

Experimental

Labeling of Mebeverine with ^{99m}Tc

Mebeverine was labeled using ^{99m}Tc by a reduction reaction using $SnCl_2.2H_2O$ (Essa et al., 2015; Richardson et al., 1977). The reaction was applied in an evacuated vial to study the parameters influencing the radiochemical yield of ^{99m}Tc-Meb to attain the optimal condition. Different concentrations of Mebeverine were used (50-300μg) followed by $SnCl_2$ with a concentration (50-300μg), the reaction pH was controlled by adding 0.1ml of variable pH system (2-11). Finally, an amount of 0.1ml (7.2MBq) of ^{99m}Tc-pertechnetate recently eluted by a molybdenum generator was added. The reaction vial was left for different breaks of time (5min-60min) by changing the reaction temperature from room temperature to 80ºC. The radiochemical yield (RCY) was calculated using paper chromatography for elution.

Radiochemical purity and yield

Measurements of the RCY and purity of the product were performed using paper chromatography and HPLC.

Paper chromatography: The RCY of ^{99m}Tc-Meb was calculated, the elution in acetone was performed to calculate free pertechnetate which was motivated to the front while the reduced colloids stayed at the starting point with the labeled mebeverine. However, the elution using saline was used to distinguish the reduced colloids from the complex. After calculating the concentration of the free pertechnetate and colloids, the amount of the ^{99m}Tc-Meb was defined from the difference (Ibrahim & Attalah, 2012).

HPLC analysis: The RCP of ^{99m}Tc-Meb was defined by HPLC. The column used was a C_{18} 5μm column (4.6mm × 150mm, Waters). The mobile phase consists of 50 mM KH_2PO_4, acetonitrile, and tetrahydrofuran (THF) (63:35:2; v/v/v) which was eluted at a flow rate of 1 ml/min. The UV detector was at λ 263nm. Separation was applied at room temperature (Souri et al., 2014). The radio-HPLC analysis was accomplished to confirm the radiochemical purity of the radiotracer.

Biological distribution

The tracer was introduced in normal mice through the intravenous route to evaluate its biological behavior. In each trial, four mice were used. The mice were sacrificed after different time intervals after injecting the tracer. All the organs were weighed using a sensitive balance and measured with a gamma counter. The injected dose was considered per gram (ID/gram). Blood, bone, and muscles were estimated to be 7%, 10%, and 40% of the whole body weight, individually (Sanad & El-Tawoosy, 2013; Challan & Massoud, 2017).

The bioevaluation of ^{99m}Tc-Meb in tumored mice was also under study; the solid tumor was evoked in mice by injecting in the right thigh 0.2ml solution of a parent tumor line (Ehrlich ascites carcinoma) diluted with sterile physiological saline, however the left thigh muscle was left untouched and taken as control (Ibrahim et al., 2018), the animals were left for 2 weeks under organized circumstance till the tumor was prompted. The tracer was injected into the tumored mice to study the prospective use of the tracer as a tumor marker. The mice were distributed to groups, 4 animals each. At the last of each experiment, the animals were sacrificed at

different intervals of time after the injection of the tracer.

Results and Discussion

Labeling of Mebeverine with ^{99m}Tc

Influence of pH reaction

The pH is one of the most important factors affecting the yield dramatically as shown in Fig. 2. A high yield of 97% was achieved at pH 4, any further shifting of the pH towards the neutral region the radiolabeling yield declined to 80%, on the other hand in the alkaline medium the yield decreased radically to 64%.

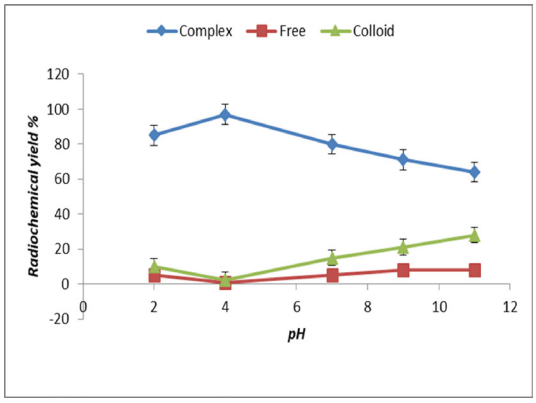

Fig. 2. Variation of radiochemical yields of ^{99m}Tc -Meb.as a function of pH [300µg Meb+ 100µL buffer at different pH+200µg $SnCl_2$] at room temperature within 30min

Influence of $SnCl_2$ amount

The effect of the amount of the reducing agent on the radiochemical yield of ^{99m}Tc -Meb was understudy as any change in its amount can affect the yield intensively. ^{99m}Tc -Meb reached 97% as the reducing agent amount reached 200µg as presented in Fig. 3. Regardless any increase in the tin amount up to 300 µg, the amount of the colloid increased while the complex amount decreased.

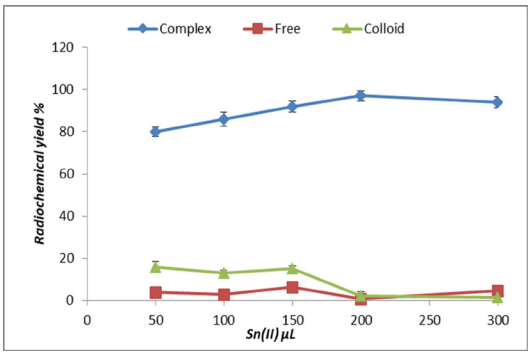

Fig. 3. Variation of the radiochemical yield of ^{99m}Tc –Meb [300µg Meb +100µL buffer pH4+Xµg $SnCl_2$] at room temperature within 30min

Influence of Mebeverine amount

The optimum amount of mebeverine needed in the reaction was studied to prevent any byproduct. A small amount of Mebeverine resulted in low RCY as the amount of ligand was not enough to combine with the reduced technetium (Motaleb, 2007). The ideal RCY (97%) was achieved at 200µg mebeverine, but then again any increase in its amount, the yield was not improved as exposed in Fig. 4.

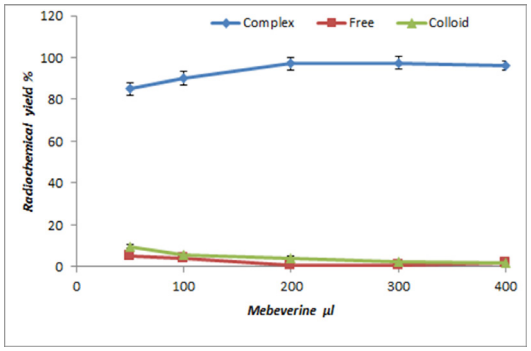

Fig. 4. Variation of the radiochemical yield of ^{99m}Tc -Meb [Xµg Meb +100µL buffer pH4+200µg $SnCl_2$] at room temperature within 30min

Influence of reaction time

The reaction time seemed to be a critical factor influencing the amount of the obtained complex. It was observed that a small reaction time was insufficient to produce the complex causing a low yield (Rashed et al., 2016). While increasing the reaction time by reaching 30min, we obtained an optimal yield of 97%. Any further increase of the reaction time did not affect the yield, as presented in Fig. 5.

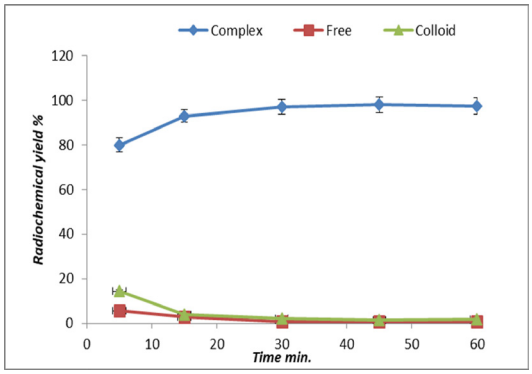

Fig. 5. Variation of radiochemical yield of ^{99m}Tc -Meb [200µg Meb +100µL pH4+200µg $SnCl_2$] at room temperature within X min

Temperature influence

The reaction temperature was an important factor affecting the yield affectedly. All data are clarified in Fig. 6 showing an optimum RCY 97˚C at ambient room temperature. Any increase in the temperature caused a slight decrease in the complex yield. The time was fixed at 30min in all the reactions.

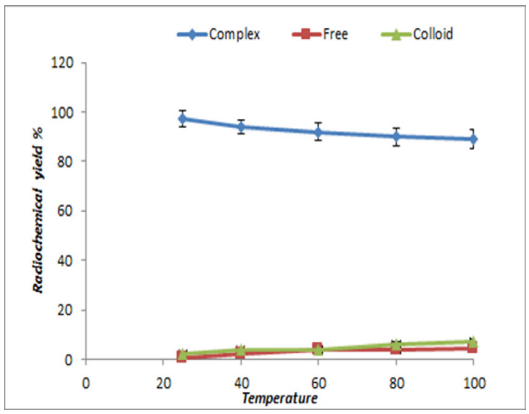

Fig. 6. Variation of radiochemical yield of ^{99m}Tc -Meb. [200µL Meb +100µL buffer pH4+200µL $SnCl_2$] at X temperature within 30min

In-vitro stability of ^{99mTc}-Mebeverine

The stability of the complex was an important factor to prevent the accumulation of any undesired by-product from the breakdown of the complex in the target or non-target organ. The in-vitro stability gives information about the best time to diagnose the patient. The ^{99m}Tc -Meb stability in serum was tested as illustrated in Fig. 7, ^{99m}Tc –Meb was stable in the serum for 12h, after that time the stability of the tracer in the serum dropped from 97% to 85%.

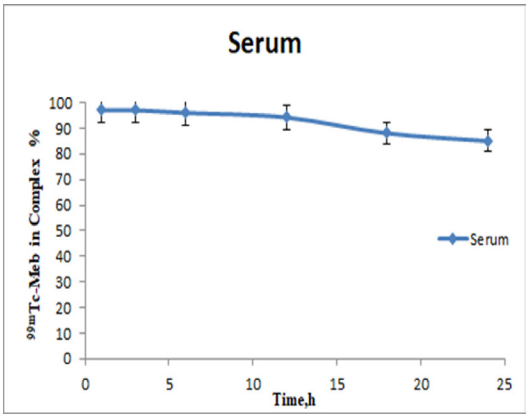

Fig. 7. Stability of ^{99m}Tc-Meb in fresh serum against time

HPLC analysis

The ^{99m}Tc –Meb was eluted at 6 min. while free ^{99m}Tc O4- was eluted at 3 min., as presented in Fig. 8. one single peak for ^{99m}Tc –Meb was obtained indicating that the complex was unpolluted without any ^{99m}Tc impurities. Likewise, the retention time of the unlabeled Mebeverine was predicted using a UV detector (Rt= 5.5min.).

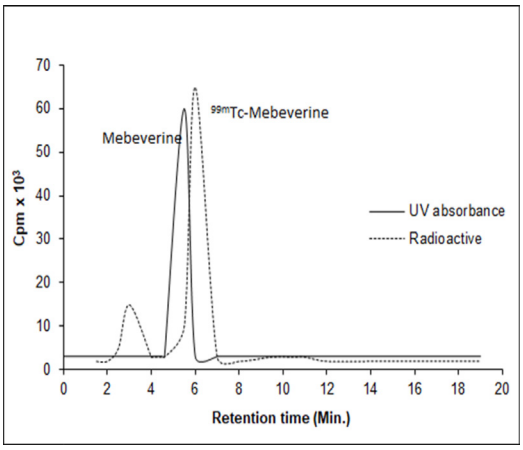

Fig. 8. HPLC analysis of ^{99m}Tc-Meb complex

Biodistribution

Figure 9 provides the data on the performance of the tracer in normal mice. The blood uptake reached at 30 min. post-injection 12%; this high ratio may be credited to the high binding of Mebeverine with plasma protein (Mayur & Avani, 2011; El Nabarawi et al., 2017). The intake of the liver reached 5 % at 30 min. post-injection, followed by a very slight decrease to 4.8% at 3h post-injection. The tracer collected in urine was 34% after 3h of the injection of the tracer, a high ratio was attributed as the kidney is the main route of excretion for the drug (Stockis et al., 2002). The uptake by the stomach reached 7.2%. The ratio of the tracer in the muscle was 5.5% at 30min. post-injection showing an increase up to 8% after 3h of the tracer injection.

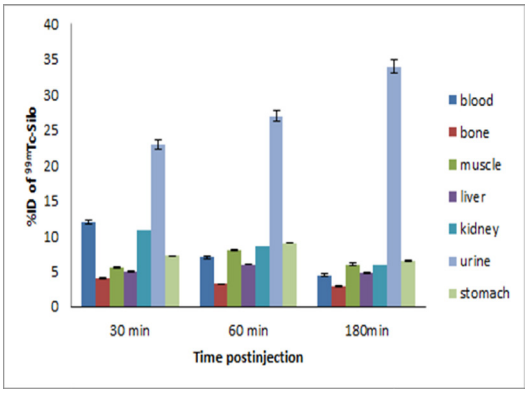

Fig. 9. Biodistribution of ^{99m}Tc-Meb in normal mice

On the other hand, the biodistribution of ^{99m}Tc-Meb in tumored mice was understudy, data is obtainable in Fig. 10. The uptake by the tumor was 14.2% after 30min. of injection and increased to 18.5% at 1 h post-injection, showing a slight decline after 3 hours, that washout was referred to as the extreme extravasation in the tumored muscle (Yamamoto et al., 2001; Harrold et al., 2013). By the use of the student's unpaired test ($P< 0.05$), the difference between the intake of the tumor and the normal muscle was broad. The Tumored (Left thigh) /Non-Tumored (right thigh) ratio (T/NT), is a very essential parameter to evaluate the selectivity and sensitivity of the tracer to the induced solid tumor (Sakr et al., 2012; Sakr et al., 2014). After 30min. post-injection, the T/NT ratio was 2.59 as presented in Table 1. The ratio was 3.14 at 180min post-injection, this value shows the high selectivity and affinity of ^{99m}Tc-Meb to a solid tumor. This good preclinical ratio specifies that ^{99m}Tc-Meb is a likely solid tumor marker.

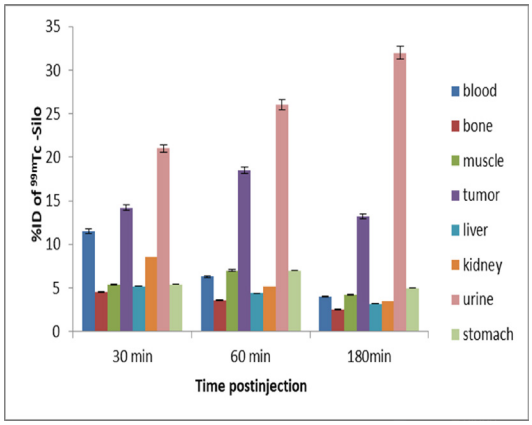

Fig. 10. Biodistribution of ^{99m}Tc-Meb in tumored mice

TABLE 1. Ratio of Tumored to Non-Tumored Muscle (T/NT)

Organs & body fluids	Detected dose/gram percent at different time intervals post injection (min)		
	30min	60 min	180min
Muscle (NT)	5.4±0.4	7±0.5	4.2±0.6
Tumor (T)	14±1.5	18.5±1.4	13.2±0.9
T/NT	2.59	2.64	3.14

Conclusion

A high RCY (97%) was obtained by labeling Mebeverine with ^{99m}Tc through a reduction reaction using $SnCl_2$. The tracer showed stability for 12h in serum. The utility of the tracer as a possible solid tumor marker was clarified by a bioevaluation of ^{99m}Tc -Meb in tumorized mice. The T/NT ratio was also calculated, reaching 3.14 at 180min post-injection which proves the high sensitivity of ^{99m}Tc-Meb to solid tumors.

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