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Preparation of ^{186}Re-Cefixime as a Potential Diagnostic and Therapeutic Agent for Bacterial Infection

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SEVERAL factors that influence the preparation of ^{186}Re-Cefixime such as the amount of Cefixime and stannous chloride, pH, reaction time and reaction temperature were studied to optimize the labeling conditions to obtain the highest radiochemical yield of ^{186}Re-Cefixime. The radiochemical purity of Rhenium-186 was determined by paper chromatography, while the radiochemical yield and purity of ^{186}Re-Cefixime were determined by electrophoresis and high-performance liquid chromatography (HPLC). The maximum radiochemical yield of ^{186}Re-Cefixime was obtained (96±2.8%) using 2mg Cefixime, 0.3mg carrier added ^{186}Re and 0.5mg stannous chloride at pH 5.5, within 30min at room temperature. The bio-distribution was carried out on three types of mice (normal, sterile infected and bacterial infected). The results show that ^{186}Re-Cefixime is more concentrated in the bacterially infected muscle (septic inflammation) than in the sterile infected muscle (aseptic inflammation). Therefore, ^{186}Re-Cefixime could be used to differentiate between septic and aseptic inflammation.

Keywords: Cefixime, Labeling, Rhenium-186, Biodistribution, Septic and aseptic inflammation.

Introduction

Physiological imaging of bacterial infection is the advantage that favors the nuclear medicine technique to determine the bacterially infected areas (Boerman et al., 2006). The radioactive nuclides labelled with biomolecules, evaluating their distribution in human bodies by SPECT or PET system, which is based on the decay mode of radionuclide (γ- or β^+- emissions), for example, ^{67}Ga-citrate as a SPECT imaging or ^{18}FDG as a PET imaging are used to depict inflammations. Several radiolabeled agents bind in vivo specifically to bacterial cells have been developed, such as a complex of ^{99m}Tc with ciprofloxacin (Unni et al., 2001 and Corstens et al., 1999), as well as ^{99m}Tc-labeled antimicrobial peptides such as ^{99m}Tc-UBI 29–41 (Hall et al., 1998), for diagnostic purposes (SPECT). Radiotherapy can be directed by diagnostic SPECT or PET (diagnostic imaging), which are merged into theranostics approaches for the diagnosis and treatment of a variety of tumor types that are rapidly gaining momentum, for example, ^{177}Lu-labeled sulfadiazine used as a possible theranostic agent for deep-seated bacterial infection (Naqvi1 et al., 2017).

^{186}Re is an ideal candidate for radioimmunotherapy, especially in bone pain palliation (Kinuya et al., 2003, 2005 and Postema et al., 2003), due to its short range β^-emission (< 2mm in tissue) with energies at 1.07 and 0.933MeV (71 and 22%, respectively), low-abundance (9%) γ-ray emission at 137keV, which allows for in-vivo tracking of the radiolabeled biomolecules and the estimation of dosimetry calculation. The suitable half-life (3.7-day) allows sufficient time for the synthesis, shipment of potential radiopharmaceuticals and the radiotherapy. It can be produced by different ways, the dominant routes are; nuclear reactor through (n, γ) reaction and cyclotron through ^{186}W(p,n)^{186}Re reaction. The principal drawback of the former reaction is that the radionuclide ^{186}Re is produced as a carrier added form with a low specific activity, whereas the latter is better due to its carrier-free nature and also for high specific activity that is generally required for the radiolabeling of tumor-specific antibodies (Volkert et al., 1991).

Cephalosporins are antibiotics of β-lactam rings widely used for the therapy of various infections in both humans and animals due to

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their antibacterial and pharmacokinetic properties. They are among the safest antibiotics to be active against penicillin-resistant bacteria and being feasible for penicillin-allergic patients. Cefxime (CEF) is a semisynthetic third-generation oral antibiotic belonging to the cephalosporin group and works by inhibiting bacterial cell wall synthesis. Hence, cefixime belongs to a class of medications called cephalosporin antibiotics. Its bactericidal action is mainly due to the inhibition of the final transpeptidation step of the peptidoglycan synthesis in the bacterial cell wall, thus inhibiting cell wall assembly resulting in bacterial cell death (Graham, 2005). Thermal stability of Cefixime was studied and confirmed its stability up to 80°C for about 3hr (Dhara et al., 2017).

This study concerns the preparation of ^{186}Re-Cefixime that can be used as a theranostic agent for bacterial infection. The affecting factors the radiochemical yield (RCY) of ^{186}Re-Cefixime such as the amount of Cefixime, pH values, reaction time, reaction temperature and the stannous chloride to optimize the labeling conditions were studied. The biodistribution of ^{186}Re-Cefixime was carried out on three types of mice (normal, sterile infected and bacterial infected) in order to demonstrate the importance of ^{186}Re-Cefixime in the distinction between septic and aseptic inflammation and pointing out its importance as a radiotherapy.

Experimental

Materials and instruments

All reagents used in the present work were of analytical grade. Cefixime was purchased from Pharco Pharmaceutical Company Alexandria, Egypt. Stannous chloride dihydrate [$SnCl_2 \cdot 2H_2O$, M.W. 225.64], was purchased from Sigma Chemical Company, USA. Natural rhenium in oxide form (A.R. Rhenium (VII) oxide, Re_2O_7 (M.W= 484.4), with chemical purity ≥ 99.9) was purchased from Aldrich Chemical Company, Germany.

Gamma-scintillation counter: Scaler Ratemeter, SR7 type, fitted with a well-type NaI(TI) crystal detector.

Ionization chamber: Capintec Radioisotope Calibrator, Model CRC 12R, USA, was used for calibrating the activity of ^{186}Re in mCi and/or GBq.

Electrophoresis apparatus EC 3000 p-series programmable (E.C. Apparatus Corporation, USA) was used to evaluate the radiochemical yield of ^{186}Re-Cefixime using Whatman paper sheet.

Preparation of ^{186}Re

Natural rhenium in oxide form (A.R. Rhenium (VII) oxide, Re_2O_7 (M.W= 484.4), of chemical purity ≥ 99.9) was prepared as a target material. The Re_2O_7 (0.1g) was wrapped in a small piece of thin aluminum foil that was previously cleaned with acetone and air-dried. The wrapped sample was placed in an aluminum can, sealed and tested for leak proof before irradiation. The target was irradiated ~ 4hr in the 22MW water-cooled Egyptian Research Reactor (ETRR-2) with a thermal neutron flux of ~10^{14} n cm^{-2} s^{-1}. Before chemical processing, the irradiated sample was cooled for ~6 d after the end of irradiation for decay of ^{188}Re ($T_{1/2}$= 16.9h). The irradiated rhenium oxide target was dissolved in 23ml double distilled water and measured by isotope calibrator to be ~2.33 mCi^{186}Re /mg Re (~86.3 MBq/mg).

Radiochemical purity of rhenium-186

Whatman paper No.3 as a stationary phase and acetone as a development medium were used. Figure 1 shows that the retardation factor (R_f) was obtained at 0.9-1, which corresponds to the perrhenate form ($^{186}ReO_4^-$) (Nomando, 2001 and Eckelman & Levenson, 1977).

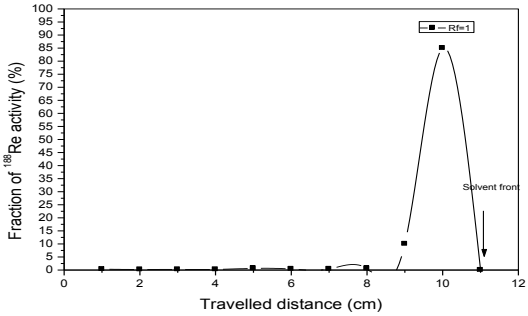


Fig. 1. Radiochromatogram of the dissolved ^{186}Re from the irradiated Re_2O_7, using Whatman no.1 ascending paper chromatographic method and acetone as a developing solvent.

Affecting factors on the RCY of ^{186}Re-Cefixime

The RCY of ^{186}Re-Cefixime was obtained by studying different factors such as different amounts of Cefixime (0.5- 5mg) in 500µL ethanol: distilled water (1:1 v/v), (0.01- 3mg) freshly prepared deoxygenated stannous chloride dehydrate in 50µL, sodium perrhenate (~37MBq of ^{186}Re), then 500µL phosphate buffer (pH 3 - 8), the reaction temperature (25-100°C) and the reaction time (10-240min).

A maximum RCY of ^{186}Re-Cefixime was obtained by adding 2mg Cefixime in 500µL ethanol: distilled water (1:1 v/v), 50µL of freshly prepared deoxygenated stannous chloride dihydrate (0.5mg), ^{186}Re (100µL, ~37MBq of ^{186}Re) and 500µL phosphate buffer (pH 5.5) within 30min at room temperature.

Determination of the radiochemical yield, chemical stability, and purity of ^{188}Re-Cefixime

Electrophoresis analysis

The reaction mixture (10µL) was placed 12cm away from the cathode. Normal saline solution (0.9% w/v NaCl solution) was used as an electrolyte solution and then the electrophoretic paper was run out for 1.5h at 300V. After that, the paper was removed, dried, cut into segments of 1cm, and counted in a well-type γ-counter. The radiochemical yield was determined by the following equation:

$$\text{Radiochemical yield (\%)} = \frac{\text{Radioactivity of } ^{186}\text{Re–Cefixime peak X 100}}{\text{Total activity}}$$

The electrophoresis technique was used to determine the radiochemical yield of ^{186}Re–Cefixime, show migration both of the perrhenate and ^{186}Re–Cefixime towards the anode at 7 and 4cm, respectively, as shown in Fig. 2.

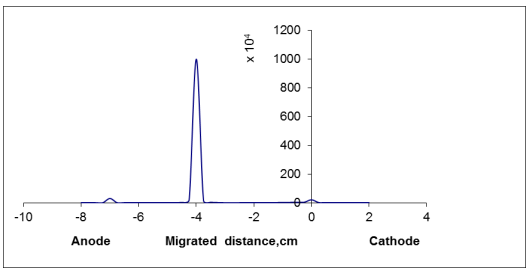


Fig. 2. Radiochromatogram of the ^{186}Re-Cefixime electrophoretic analysis.

HPLC analysis

The radiochemical purity and chemical stability of ^{186}Re-Cefixime were determined by its injection (5–10µl) to HPLC. A reversed phase column-18 (250mm × 4.6 mm, 5µm) as a stationary phase, methanol: H_2O (70: 30 v/v) as a mobile phase at a flow rate of 1ml/min, and UV region (at the wavelength 254nm), were used for the determination of Cefixime and perrhenate (ReO_4^-). The radioactivity of ^{186}Re-Cefixime was determined by using a well-type NaI (Tl) crystal connected to single channel analyzer.

Biodistribution

Injection of the ^{186}Re-cefixime tracer

^{186}Re-Cefixime (100µL, 2.5-3MBq) was injected intravenously (I.V.) into the tail vein of the mice. Groups of four mice were used for each experiment. The mice were sacrificed by the decapitation under chloroform anesthesia at 15, 60, 120 and 240min post injection. Blood samples were collected at the time of decapitation. Both thighs (the right thigh muscle as a target and the left thigh muscle as a control), all body organs were dissected, weighed and counted their radioactivity using a well-type NaI(Tl) detector connected with a single channel γ-counter (SR-7). Results were expressed as percent of the injected dose per organ or body fluid. Bone, blood and muscles were calculated as 10, 7, and 40% of the total body weight, respectively (Korde et al., 1998). The uptake of ^{186}Re-Cefixime in muscles or organs was calculated as a percentage of the injected dose per 1g of the body weight.

Bacterial infection and Sterile Inflammation

Infections were introduced by injection of 2x 10^6 by *E. Coli* (Sakr, 2010; Johannsen & Spies, 1991 and El-Ghany et al., 2005), suspended in 0.1ml saline into the right thigh muscle. Five days later, the growth appeared. Sterile inflammation was induced by the intramuscular injection of the autoclaved turpentine oil into the right thigh muscle 0.1ml/mice. Six days later, the growth appeared. Target to non-target (T/NT) ratio was calculated at different interval times for the uptake of ^{186}Re-cefixime in inflamed muscle to the control muscle and shown in Tables 1, 2 and 3 to clarify the difference and to evaluate the usefulness of ^{186}Re-cefixime to distinguish between the different types of inflammations.

Results and Discussion

Effect of cefixime amount

The RCY of ^{186}Re-cefixime as a function of cefixime concentration in the presence of stannous chloride dihydrate as a reducing agent was studied as shown in Fig. 3. The results reveal that the RCY of ^{186}Re-cefixime increased from 85±1.7 to 96±2.8% by increasing the amounts of cefixime from 0.5 to 5mg. An increasing cefixime amount more than 2mg does not affect the RCY of ^{186}Re-cefixime while, less than this amount leads to decreasing the RCY that may be attributed to the fact that the concentration of cefixime is insufficient to shift completes the complex formation towards the final complex (Amin et al., 2009).

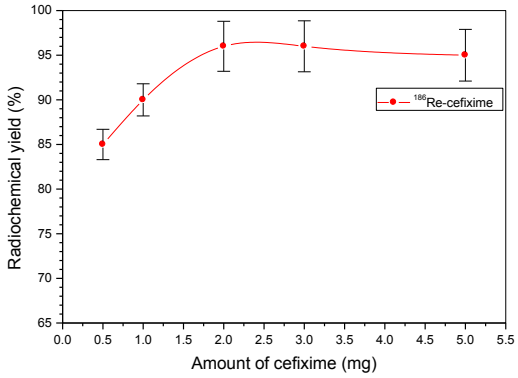


Fig. 3. The radiochemical yield of ^{186}Re-Cefixime as a function of Cefixime amount [100µL (37MBq) $^{186}ReO_4^-$, 400µL of Cefixime (xmg) in distilled water: ethanol (1:1 v/v), 500µL phosphate buffer (pH 5.5), 50µL (500µg) stannous chloride] at 25°C within 30min reaction time.

Effect of stannous chloride dihydrate amount

The amount of stannous chloride on the RCY of ^{186}Re-cefixime was studied in Fig. 4. The RCY of ^{186}Re-cefixime increased from 75.6±1.5 to 96±2.8 % by increasing the amount of stannous chloride ($SnCl_2.2H_2O$) from 0.01 to 0.5mg, respectively. The amount of stannous chloride less or greater than 0.5mg, such as 0.01 or 3mg leads to the RCY of ^{186}Re-cefixime decreasing to 75.6±1.5 or 60±1.2%, respectively. The RCY of ^{186}Re-Cefixime decreased in the low amount of $SnCl_2$ that may be attributed to its insufficient concentration to reduce the rhenium (VII) (ReO_4^-) to lower oxidation states (El-Kawy & Talaat, 2016). While most of the ligand molecule was consumed in the formation of complex, the perhenate is reduced to insoluble rhenium (IV) $ReO_2.xH_2O$ in the absence of ligand at the high concentration of stannous chloride (Azmi et al., 2013). Moreover, at a high concentration of Sn(II), the reduction reaction rate increases to form a colloid so that it becomes more competitive with respect to the complexation reaction, thus decreasing the RCY of ^{186}Re-cefixime.

Effect of pH of the reaction mixture

Figure 5 shows the obtained results by the preparation of ^{186}Re-cefixime at different values of pH. The RCY decreased to 75±1.5 % at pH 2 that may be attributed to the protonation of Cefixime, and this may lead to decreasing the stability of the ^{186}Re-Cefixime complex. Increasing the pH from 2 to 5.5 increases the RCY from 75±1.5 to 96±2.8%, this may be attributed to the deprotonation of cefixime and may increase the stability of the ^{186}Re-cefixime complex. Then, the RCY of ^{186}Re-cefixime drastically decreased to be 60±1.2 % at pH 8, which may be attributed to the formation of stannous hydroxide ($Sn(OH)_3$ (El-Kawy & Talaat, 2016).

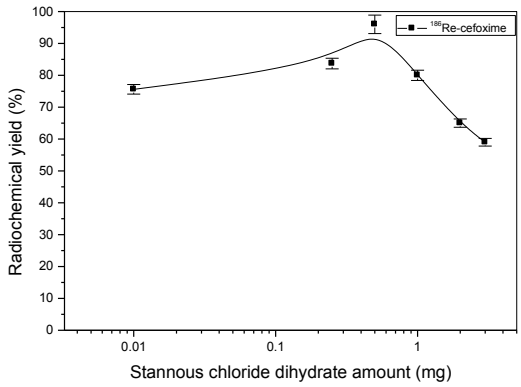


Fig. 4. The radiochemical yield of ^{186}Re-Cefixime as a function of stannous chloride concentration [100µL (37MBq) $^{186}ReO_4^-$, 400µL of Cefixime (2mg) in distilled water: ethanol (1:1 v/v), 500µL phosphate buffer (pH 5.5), 50µL (xmg) stannous chloride] at 25°C within 30min reaction time.

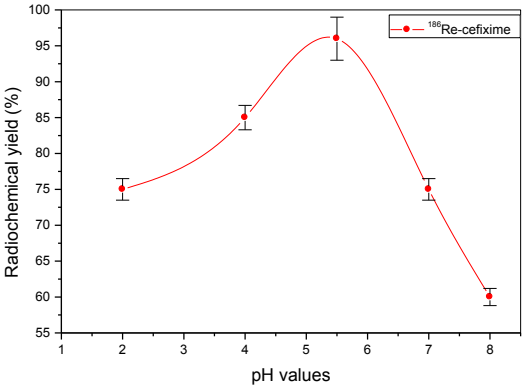


Fig. 5. The radiochemical yield of ^{186}Re-Cefixime as a function of pH value [100µL (37MBq) $^{186}ReO_4^-$, 400µL of Cefixime (2mg) in distilled water: ethanol (1:1 v/v), 500µL Phosphate buffer variable pH , 50µL (0.5mg) stannous chloride] at 25°C within 30 min reaction time.

Effect of reaction temperature

The maximum RCY of ^{186}Re-cefixime (96±2.8%) was obtained at the room temperature - up to 40°C, then the RCY decreased to 87±2.1, 75±1.5 and 65±1.2% by increasing the reaction temperature to 60, 80 and 100°C, respectively, as shown in Fig. 6. The results showed that the room temperature represents the optimum temperature used in the preparation of ^{186}Re-cefixime

complex. The RCY of ^{186}Re-cefixime decreased by increasing the temperature, this may be due to the fact that the temperature disintegrates the ^{186}Re-cefixime complex, but it does not affect the cefixime as a compound. This explanation is more realistic because the literature data confirmed the thermal stability of cefixime up to 80°C (Dhara al., 2017).

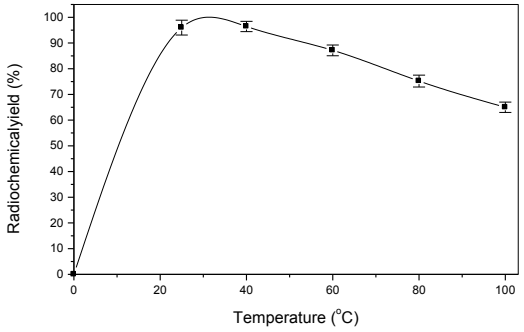


Fig. 6. The radiochemical yield of ^{186}Re-cefixime as a function of reaction temperature [100μL (37MBq) $^{186}ReO_4^-$, 400μL of Cefixime (2mg) in distilled water: ethanol (1:1 v/v, 500μL phosphate buffer (pH 5.5), 50μL (0.5mg) stannous chloride] at 30min and different reaction temperatures.

Effect on reaction time

The effect of reaction time on the RCY of ^{186}Re-Cefixime was shown in Fig. 7. The RCY of ^{186}Re-Cefixime increased from 79±1.6 to 96±2.8% and reached an equilibrium by increasing the reaction time from 10 to 30min at room temperature. The ^{186}Re-cefixime was stable for up to 4hr.

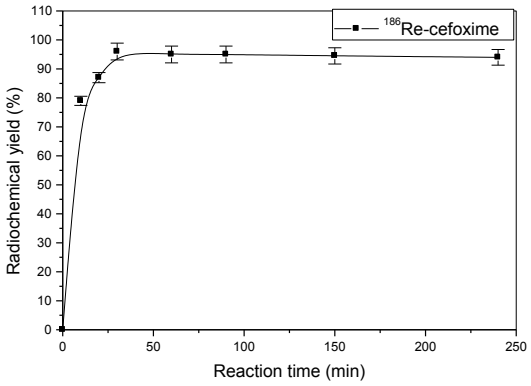


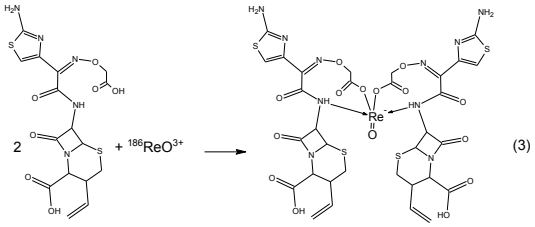
Fig. 7. The radiochemical yield of ^{186}Re-Cefixime as a function of reaction time [100μL (37MBq) $^{186}ReO_4^-$, 400μL of Cefixime (2mg) in distilled water: ethanol (1:1 v/v), 500μL phosphate buffer (pH 5.5), 50μL (0.5mg) stannous chloride] at 25°C and different reaction time.

Reaction mechanism

The maximum RCY of ^{186}Re-Cefixime (96±2.8%) was obtained by reacting Cefixime 2mg (4.4×10^{-6}μmoles) with ^{186}Re-carrier added 0.43mg (2.3×10^{-6}μmoles) in the presence of stannous chloride dihydrate 0.5mg (2.2×10^{-6} moles) at room temperature and pH 5.5 within 30min. The reactions were carried out depending on the molar ratio 2: 1: 1 of Cefixime: Rhenium: Stannous, as in equations 1-3 and based on the active groups, which has been used to form Pd(II)-cefixime complex (Azmi et al., 2013).

$$Sn2+ \leftrightarrow Sn4++2e- \quad (1)$$

$$^{186}ReO_4^- + 6H^+ + 2e^- \leftrightarrow {}^{186}ReO^{3+} + 3H_2O \quad (2)$$



Purity and in vitro stability of ^{186}Re-Cefixime

^{186}Re-Cefixime was separated by HPLC for the in vivo study. Figure 8 shows that the retention times of the free perrhenate, Cefixime, and ^{186}Re-Cefixime are 4, 11 and 12min, respectively. It shows that the Cefixime and ^{186}Re-Cefixime are stable due to the absence of any peaks that resulted from the decomposition of Cefixime or ^{186}Re-Cefixime. The literature data show the decomposition of Cefixime using the C18 column at 254nm (Adam et al., 2011). The RCY of ^{186}Re-Cefixime reached the equilibrium state within 30min and its chemical stability lasts up to 240min (Fig. 7).

Biodistribution studies

In many cases, there is a difficulty in discriminating between sterile inflammation (aseptic) as in head traumas, accidental traumas, joint, bone or muscle and bacterial inflammation (septic), so the sequence of treatment may not be started well. Hence, the patient may receiveuseless drugs. SPECT radio-pharmaceutics could be used to distinguish between both cases and so serve patients and medications. Many trials were performed using several labeled compounds such as ciprofloxacin, norfloxacin, ceftriaxone and others (Zolle, 2007 and El-Ghany et al., 2005).

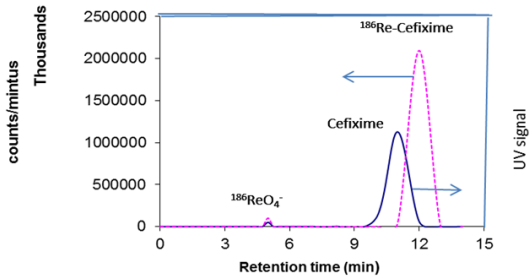


Fig. 8. High performance liquid chromatography elution profile of ^{186}Re, Cefixime and ^{186}Re-Cefixime separated on reversed phase column nucleosil (250mm X 4.6mm, 5μm) at a flow rate of 1ml/min.

In normal mice

Table 1 presents the data collected from the injection of ^{186}Re-cefixime in the tail vein of mice. Liver uptake of ^{186}Re-cefixime (12.2% at 120min post-injection) was due to the high blood vasculature and the lipophilicity of the tracer. The radioactivity located in the kidney increased from 12.4 to 20.2% by increasing the time from 60 to 120min post-injection. The uptake of ^{186}Re-cefixime in the corresponding organs (non-targets) of the studied mice (normal, steril infection and bacterial infections) are much closer to each other, such as the uptake of the stomach which was 3.7, 3.9 and 3.1%, respectively at 120min post-injection.Whereas a variation between the muscles uptake (target) (1.1, 1.8 and 6.2%, respectively at 120min post-injection) was observed. The ^{186}Re-cefixime was removed from the circulation mainly via the kidney and liver with an average 20 and 13%, respectively.

In sterile inflamed mice

The bio-distribution of ^{186}Re-cefixime in sterile inflamed muscle in Table 2 shows a slight difference compared to that in the control mice. The ^{186}Re-cefixime uptake increased in the sterile inflamed muscle compared to the muscle control that may be attributed to the high vascularity leading to the vasodilatation (inflammation site) and hence a high blood flow in this site (non-specific uptake). This is also clear from T/NT in Table 2. Sterile inflammation sites are rich with a Cyclooxygenase enzyme ($CoX_{1,2}$) which makes orientation to drugs inhibit ($CoX_{1,2}$) as analgesics or anti-inflammatories not antibiotics as cefixime (Yoshimi et al., 1997).

In infected mice

Table 3 shows the ratio of target to non-target (T/NT). The T/NT ratios of the sterile infected muscle differ from the bacterially infected muscle at all times recorded (15, 50, 120 and 240min). The maximum ratio of T/NT (3.9) was obtained at 120min post-injection for the bacterially infected muscle. Figure 9 demonstrates that the accumulated activity of ^{186}Re-cefixime in bacterially infected muscle was fourfold the control muscle at 120min. All data supported the location of ^{186}Re-cefixime in the infected muscle due to bacteria.

In vivo stability of ^{186}Re-Cefixime

The ^{186}Re-Cefixime was injected and studied in mice at different time intervals (15, 60, 120 and 240min). The results confirmed that the compound achieved the desired goal, its concentration in the infected muscle due to bacterial orientation (Tables 1-3). The results clarified that the radioactivity has not exceeded 4% in the stomach and this means that the ^{186}Re-Cefixime does not decompose to perrhenate (ReO_4^-) up to 240min. Literature data referred to the increase of radioactivity level in the stomach (40-50%) is an indicator for the decomposition of thelabeled compound (Zucier et al., 2004).

Conclusion

The pH plays a vital role in the ^{186}Re-cefixime preparation, where a maximum RCY (96±2.8%) was obtained in a weak acid (pH 5.5) and in the presence of stannous chloride as a convenient reducing agent that reduces ^{186}Re(VII) to a lower oxidation state such as ^{186}Re(V), which is easily introduced into the cefixime to form oxo-core complex. The strong acidity and alkalinity media lead to the decreasing of RCY of ^{186}R-cefixime. Firstly, the formation of unstable ^{186}Re-cefixime could be obtained at a low pH. Secondly, the deprotonation of the cefixime at a high pH, definetly decreases the stability of the ^{186}Re-cefixime complex. Moreover, increasing the hydroxide concentration could be responsible for the partial hydrolysis of the complex. Biodistribution studies clarified that the ^{186}Re-cefixime is more concentrated in bacterially infected muscle (septic inflammation) fourfold the sterile infected muscle. Therefore, ^{186}Re-cefixime radiotracer is suggested to be used as a diagnostic and therapeutic agent to the bacterial infection due to the fact that ^{186}Re has a short range β^-emission (< 2mm in tissue) with energies at 1.07 and 0.933MeV (71 and 22%, respectively), low-abundance γ-ray emission (9%) at 137keV and its suitable half-life (3.7-day).

TABLE 1. Biodistribution of ^{186}Re-Cefixime in control mice

Organs & body fluids	Percent I.D./gram organ			
	Time post injection			
	15min	60min	120min	240min
Blood	17.1±1.10	13.2±0.02*	5.9±0.04*	3.4±0.04*
Bone	0.70±0.01	1.90±0.01*	1.8±0.01*	1.40±0.01*
Muscle	0.50±0.01	1.20±0.02*	1.10±0.002	1.04±0.002
Liver	14.30± 0.5	17.5±0.15*	12.2±0.16*	6.70±0.16*
Lung	3.20±0.10	5.92±0.12*	3.3±0.02*	2.30±0.02*
Heart	6.05±0.05	4.51±0.05*	3.5±0.01*	2.20±0.01*
Stomach	4.80±0.09	6.5±0.30	3.70±0.16*	2.10±0.16*
Intestine	3.10±0.50	5.4 ±0.30	6.50±0.4*	3.10±0.3*
Kidney(urine)	8.90±0.40	12.4±0.60	20.20±0.30*	01.80±0.30*
Spleen	0.90±0.02	1.60±0.04*	1.80±0.02	0.90±0.02

Values represent mean±SEM, N= 4

*: Means significantly differ from the previous each value using unpaired student's t-test P< 0.05.

TABLE 2. Bio-distribution of ^{186}Re-Cefixime in sterile inflamed mice.

Organs & body fluids	Percent I.D./gram organ			
	Time post injection			
	15min	60min	120min	240min
Blood	14.7±1.10	10.02±0.02*	5.9±0.04*	3.5±0.04*
Bone	0.70±0.01	1.90±0.01*	1.8±0.01*	1.40±0.01*
Liver	14.30± 0.5	18.80±0.15*	14.92±0.16*	6.90±0.16*
Lung	3.50±0.10	5.9±0.12*	3.9±0.02*	3.30±0.02*
Heart	6.50 ±0.05	4.5±0.05*	2.9±0.01*	1.40±0.01*
Stomach	4.80±0.09	6.1±0.30	3.90±0.16*	2.80±0.16*
Intestine	3.40±0.50	5.9±0.30	6.80±0.9*	3.70±0.19*
Kidney,(urine)	9.90±0.40	12.04±0.60	20.20±0.30*	25.80±0.30*
Spleen	0.90±0.02	1.60±0.04*	0.80±0.02	0.50±0.02
Sterile inflamed muscle	0.70±0.02	1.60±0.04*	1.80±0.02	1.50±0.02
Control muscle	0.50±0.01	1.20±0.02*	1.10±0.002	1.04±0.002
T/NT	1.4	1.33	1.64	1.44

Values represent mean±SEM, N= 4

*: Means significantly differ from the previous each value using unpaired student's t-test P< 0.05.

TABLE 3. Biodistribution of ^{186}Re-Cefixime in septic infected mice.

Organs & body fluids	Percent I.D./gram organ			
	Time post injection			
	15min	60min	120min	240min
Blood	14.5.±1.10	11.02±0.02*	6.856±0.04*	2.9±0.04*
Bone	0.65±0.01	1.90±0.01*	1.8±0.01*	1.40±0.01*
Liver	13.30± 0.5	17.80±0.15*	11.92±0.16*	6.90±0.16*
Lung	3.50±0.10	5.9±0.12*	2.9±0.02*	2.70±0.02*
Heart	6.55±0.05	4.50±0.05*	2.8±0.01*	1.50±0.01*
Stomach	4.40±0.09	6.1±0.30	3.10±0.16*	2.10±0.16*
Intestine	3.40±0.50	5.4±0.30	6.10±0.9*	3.50±0.19*
Kidney and (urine)	6.20±0.40	12.4±0.60	20.20±0.30*	23.80±0.30*
Spleen	1.10±0.02	1.60±0.04*	1.80±0.02	0.90±0.02
Septic infected muscle	1.5±0.40	4.04±0.60	6.20±0.30*	3.80±0.30*
Control muscle	0.50±0.01	1.30±0.02*	1.6±0.002	1.4±0.002
T/NT	3	3.11	3.9	2.71

Values represent mean±SEM, N= 4

*: Means significantly differ from the previous each value using unpaired student's t-test P< 0.05.

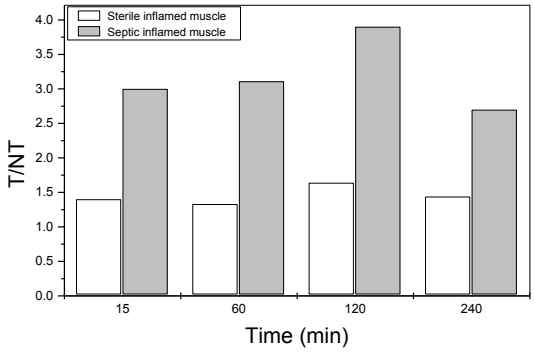


Fig. 9. The activity of infected site to non-infected site as a function of time postinjection of ^{188}Re-Cefixime into the sterile infected and the septic infected mice.

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تحضيرالسيفاكزيم المرقم بالرنيوم-186 المشع كعامل تشخيصي وعلاجي محتمل للعدوى البكتيرية

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تم دراسة العديد من العوامل التي تؤثر على تحضير السيفاكزيم المرقم بالرنيوم-186 المشع مثل كمية السيفاكزيم و كلوريد القصدير، ودرجة الحموضة، زمن ودرجة حرارة التفاعل لتحسين ظروف عملية الترقيم. حيث تم الحصول على أقصى عائد كيميائي مشع من السيفاكزيم المرقم بالرنيوم-186 (96±2.8%) باستخدام 2 مجم سيفاكزيم و 0.3 مجم حامل للرنيوم-186، و 0.5 مجم كلوريد القدير عند الاس الهيدروجيني 5.5، خلال 30 دقيقة عند درجة حرارة الغرفة. تم تحديد النقاوة الإشعاعية الكيميائية للرنيوم-186 المشع بواسطة كروماتوغرافيا ورقية، في حين تم تحديد العائد الإشعاعي الكيميائي ونقاوة السيفاكزيم المرقم بالرنيوم-186 المشع عن طريق الترحيل الكهربي وكروماتوغرافيا السائل عالى الأداء (HPLC). تم إجراء التوزع البيولوجي على ثلاثة أنواع من الفئران (عادية، مصابة معقمة ومصابة بكتيريا). وأظهرت النتائج أن السيفاكزيم المرقم بالرنيوم-186 المشع أكثر تركيزا في العضلات المصابة بكتريا من العضلات المصابة المعقمة (الإلتهاب معقم). لذلك، يمكن استخدام 186 السيفاكزيم المرقم بالرنيوم-186 للتمييز بين الإلتهابات البكتيرية والعقيمة.