

# Radioiodination of Cephalexin with $^{125}\text{I}$ and Its Biological Behavior in Mice

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## ABSTRACT

A procedure for radioiodination of Cephalexin with iodine-125 was carried out via an electrophilic substitution reaction. Using 3.7 MBq of  $\text{Na}^{125}\text{I}$ , 200  $\mu\text{l}$  of cephalexin as substrate, 200  $\mu\text{l}$  of iodogen as oxidizing agent in acetone at 40°C for 20 min, a maximum radiochemical yield of  $^{125}\text{I}$ -Ceph (95%) was obtained. The labeled compound was separated and purified by means of high-pressure liquid chromatography (HPLC). The biological distribution in normal and inflamed (septic) mice indicates the suitability of radioiodinated cephalexin for imaging of inflammation

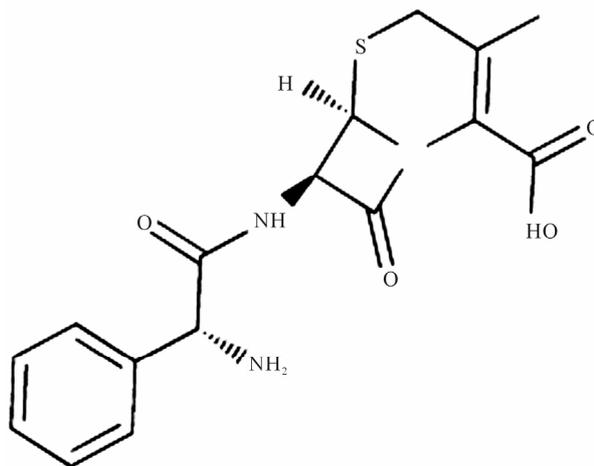
**Keywords:** Cephalexin;  $\text{Na}^{125}\text{I}$ ; Electrophilic Substitution Reaction; Radioiodination; Inflammation

## 1. Introduction

Cephalexin is a first-generation cephalosporin antibiotic, introduced in 1967 by Eli Lilly and Company [1]. Cephalexin is used to treat urinary tract [2], respiratory tract, skin and soft tissue infections and sometimes to treat acne. In addition to being a rational first-line treatment for cellulitis, it is a useful alternative to penicillins in patients with penicillin hypersensitivity. Caution should always be taken when prescribing cephalosporins to those with strong history of true penicillin hypersensitivity [3]. However, cephalexin and other first-generation cephalosporins are known to have a modest cross-allergy in patients with penicillin hypersensitivity. Cephalexin fights bacteria in the body. It works by interfering with the bacteria's cell wall formation. This weakens the cell wall, causing its rupture, and killing of the bacteria.

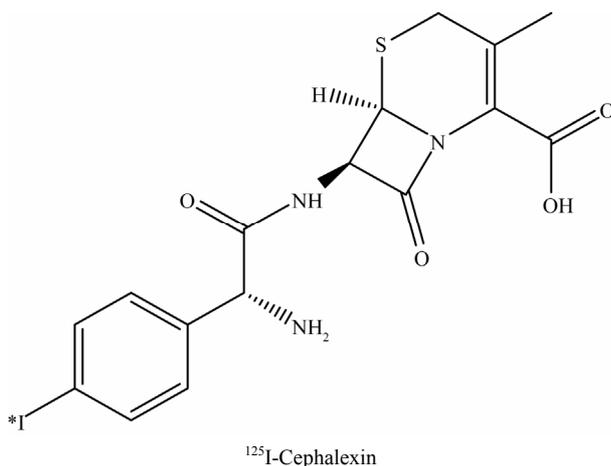
A studies of  $^{125}\text{I}$ -Labeled derivatives of the beta-lactam antibiotics cephalexin, cephadrine, cefaclor and 6-alpha-aminopenicillanic acid have been obtained by reacting these compounds with ( $^{125}\text{I}$ )-Bolton-Hunter reagent. The iodinated derivatives showed a very high affinity of binding to their target proteins with apparent half-saturating concentrations in the nano-molar range [4]. Other studies interested in the pharmacokinetics of cefaclor and cephalexin were characterized in patients with creatinine clearance ranging from 0 to 147 ml/min. Serum and urine levels of the antibiotics were measured by bioassay. For cephalexin, corresponding half-lives

were 15.4 h and 58 min, that study did present a dosage nomogram for calculating the appropriate adjustments to the loading dose based on patient weight and maintenance dose based on corrected creatinine clearance [5]. The objective of this work was oriented to label of Cephalexin with  $^{125}\text{I}$  and the factors affecting the labeling yield were investigated. The suggested structure of  $^{125}\text{I}$ -Cephalexin which formed via an electrophilic substitution reaction in the presence of iodogen as oxidizing agent; where  $^{125}\text{I}^+$  for  $\text{H}^+$  exchange in the ring is in Para-position.



Chemical Formula:  $\text{C}_{16}\text{H}_{17}\text{N}_3\text{O}_4\text{S}$   
Cephalexin

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## 2. Experimental

### 2.1. Materials and Methods

All chemicals used in the present work were of analytical grade. Cephalexin was obtained from Pharco Company, Egypt and was used without any purification.  $\text{Na}^{125}\text{I}$  (185 MBq/5 mL) in diluted NaOH, was purchased from Institute of Isotopes, Budapest, Hungary. The statistical analysis was performed using SPSS® software, USA.

### 2.2. Labeling of Cephalexin with $\text{Na}^{125}\text{I}$

The labeling reaction was carried out by the precipitation of iodogen on the wall of glass tube using chloroformic solution of iodogen. The experiment was performed by taking (25 - 300  $\mu\text{g}$ ) of cephalexin followed by (25 - 300  $\mu\text{g}$ ) of iodogen. Then 10  $\mu\text{L}$  (3.7 MBq  $\text{Na}^{125}\text{I}$ ) of NaOH solution were added and the reaction mixture was kept at different temperature for specific time. The different parameters that affect the radiochemical yield of  $^{125}\text{I}$ -Ceph were studied.

### 2.3. Radiochemical Yield and Purity

The radiochemical yield was determined by TLC and Electrophoresis while the radiochemical purity by HPLC:

#### 2.3.1. Electrophoresis Analysis

Electrophoresis was done using cellulose acetate strips. These strips were moistened with 0.02 M phosphate buffer pH 7 and then were placed in the chamber. Samples of 5  $\mu\text{L}$  were applied at a distance of 12 cm from cathode. Standing time and applied voltage were continued for 90 min. Developed strips were removed, dried and cut into 1cm segments. They were counted using a well-type NaI (TI) detector connected with a single-channel  $\gamma$ -counter.

#### 2.3.2. HPLC Analysis

The radiochemical yield% and the purity of Cephalexin

was determined by injection of 10  $\mu\text{L}$  of unlabeled drug into the column (Rp-18.  $250 \times 4 \text{ mm}^2$ , 4 - 6  $\mu\text{m}$ , Lichrosorb) built in HPLC Merck model 2010 Shimadzu model, which consists of pumps LC-9A, Rheohydrion injector (Syringe Loading Sample Injector-7125) and UV spectrophotometer detector (SPD-6A) adjusted to the 254 nm wavelength using Water: Methanol: Acetonitrile: Glacial acetic acid (50:20:30:1) as mobile phase with flow rate 1.5 ml/min [6].

## 2.4. Biodistribution Study

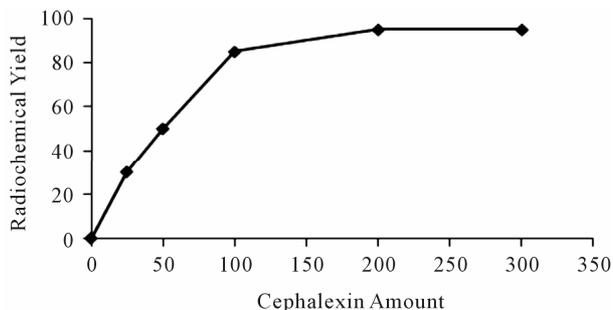
Organ distribution studies were carried out in a group of three male Albino mice. Each animal was injected in the tail vein with 0.2 ml solution containing 3.7 MBq of  $^{125}\text{I}$ -Cephalexin. The mice were put in metabolic cages for the recommended time then sacrificed. Time dependent pharmacokinetic studies were carried out at (30 min, 1 h, 2 h, and 4 h) post injection. The organs as well as other body parts and fluids were dissected. Activity in each organ was counted and expressed as a percentage of the injected activity per organ. The weights of blood, bone and muscles were assumed to be 7%, 10% and 40% of the total body weight, respectively.

## 3. Results and Discussion

### 3.1. Factors Affecting the Labeling Yield

#### 3.1.1. Effect of Substrate Amount

The radiochemical yield of  $^{125}\text{I}$ -Ceph as a function of Cephalexin amount was studied as shown in **Figure 1**. The results indicate, at low amounts of cephalexin, (25  $\mu\text{g}$  - 100  $\mu\text{g}$ ) the radiochemical yields of  $^{125}\text{I}$ -Ceph were 30% and 85%, respectively. The radiochemical yield was highly affected by increasing the amount of cephalexin to 200  $\mu\text{g}$  reaching 95%, but further increase in the amount of the substrate didn't affect the radiochemical yield. This may be attributed to the fact that the yield reaches the saturation value (95%) because the entire generated iodonium ions in the reaction are captured at that concentration of Cephalexin [7].



**Figure 1.** Variation of the radiochemical yield of  $^{125}\text{I}$ -Ceph as a function of Ceph amount [200  $\mu\text{g}$  iodogen and 10  $\mu\text{L}$  of 3.7 MBq  $\text{Na}^{125}\text{I}$ , x  $\mu\text{g}$  Ceph] at 40°C for 20 min.

### 3.1.2. Effect of Chloramine-T (CAT) Amount

Sodium iodide is the simplest and versatile source of iodinating reagent for organic molecules [8]. Elemental iodine was formed by the oxidation of sodium iodide with oxidizing agents. Chloramine-T is the most famous oxidizing agent used to produce  $\text{H}_2\text{OI}^+$  and HOI from sodium iodide [9]. The influence of CAT amount on the radioiodination of Cephalexin was studied as shown in **Figure 2**. The data clearly show that 25  $\mu\text{g}$  and 50  $\mu\text{g}$  of CAT was not sufficient to oxidize radioiodide ions in the reaction mixture [10]. By increasing the concentration of CAT to 100  $\mu\text{g}$ , the yield of  $^{125}\text{I}$ -Ceph was increased to 80%. Increasing the CAT concentration above 200  $\mu\text{g}$  leads to a decrease in the yield due to the formation of undesirable oxidative side reactions like chlorination and polymerization [11].

### 3.1.3. Effect of Iodogen Amount

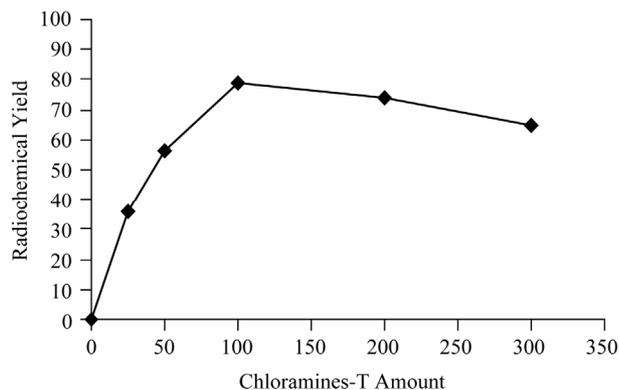
The iodination of Ceph using iodogen as an oxidizing agent was achieved by the precipitation of iodogen on the wall of glass tube using chloroformic solution of iodogen. **Figure 3** shows that the radiochemical yield of  $^{125}\text{I}$ -Ceph is low with small amount of iodogen. By increasing the concentration of iodogen to 200  $\mu\text{g}$ , the radiochemical yield increased to 95%. Any further increase in the amount of iodogen to 300  $\mu\text{g}$  didn't cause any increase in the radiochemical yield of  $^{125}\text{I}$ -cephalexin.

### 3.1.4. Effect of N-Bromo Succinimide (NBS)

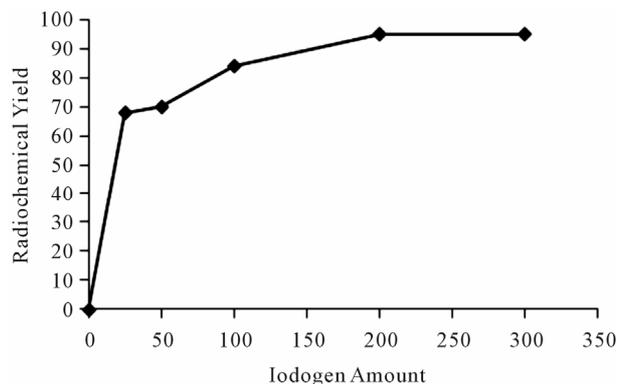
The effect of the NBS amount as an oxidizing agent was studied as shown in **Figure 4**. The data reveal that at low concentration the radiochemical yield is very low. But with further increase in the amount of NBS the radiochemical increased reaching 74% at 300  $\mu\text{g}$  of NBS.

### 3.1.5. Effect of the Reaction Temperature

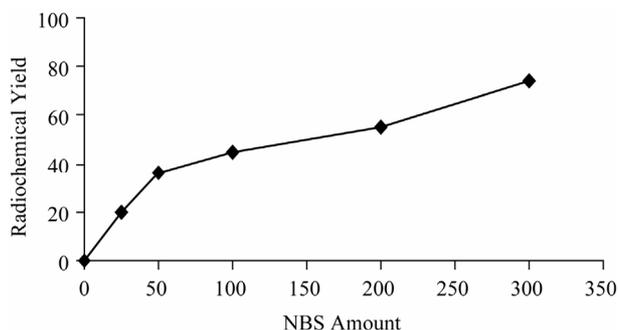
The effect of the reaction temperature (25°C - 80°C) on the radioiodination of Cephalexin by  $^{125}\text{I}$  was carried out



**Figure 2.** Variation of radiochemical yield  $^{125}\text{I}$ -Ceph as a function of Chloramine-T amount [200  $\mu\text{g}$  Ceph., (x  $\mu\text{g}$ ) CAT and 3.7 MBq  $\text{Na}^{125}\text{I}$ ] at 40°C within 20 min.



**Figure 3.** Variation of radiochemical yield of  $^{125}\text{I}$ -Ceph as a function of Iodogen amount [200  $\mu\text{g}$  Ceph, x  $\mu\text{g}$  I odogen and 3.7 MBq  $\text{Na}^{125}\text{I}$ ] at 40°C within 20 min.

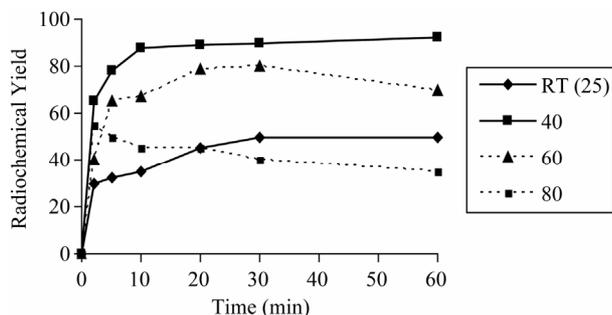


**Figure 4.** Variation of radiochemical yield of  $^{125}\text{I}$ -Ceph as a function of NBS amount [200  $\mu\text{g}$  Ceph, x  $\mu\text{g}$  NBS and 3.7 MBq  $\text{Na}^{125}\text{I}$ ] at 40°C within 20 min.

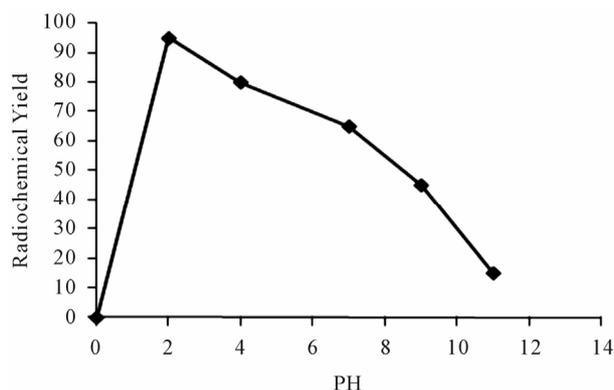
at interval time using the optimum concentration of both Ceph and iodogen (200  $\mu\text{g}$  Ceph and 200  $\mu\text{g}$  iodogen). The data indicate that the reaction temperature was found to be significant factor affecting the radiochemical yield. As shown in **Figure 5** the radiochemical yield of  $^{125}\text{I}$ -Ceph was low at room temperature all over the time. Increasing the reaction temperature to (40°C) within 10 min the radiochemical yield of  $^{125}\text{I}$ -Ceph reached to (95%) and by increasing the reaction time the radiochemical yield wasn't affected. On the other hand, increasing the reaction temperature to 60°C up to 80°C the radiochemical yield decreased. This may be attributed to the thermal decomposition of the labeled compound or distortion of the oxidizing agent [12].

### 3.1.6. Effect of pH

The effect of the hydrogen ion concentration of the reaction mixture on the radiochemical yield of  $^{125}\text{I}$ -Ceph in presence of iodogen as oxidizing agent was studied. Different buffers system at pH ranging from 2 to 11 were used. The results of this study were presented in **Figure 6**, which clearly showed that the optimum yield of  $^{125}\text{I}$ -Ceph (95%) was obtained at pH 2. This may be due to Cephalexin is an acid-stable cephalosporins [13]. When



**Figure 5.** Variation of radiochemical yield of  $^{125}\text{I}$ -Ceph as a function of reaction time [200  $\mu\text{g}$  Ceph and 200  $\mu\text{g}$  iodogen and 3.7 MBq  $\text{Na}^{125}\text{I}$ ] at different temperature.



**Figure 6.** Variation of radiochemical yield of  $^{125}\text{I}$ -Ceph as a function of pH [200  $\mu\text{l}$  Ceph, 200  $\mu\text{l}$  iodogen and  $\text{Na}^{125}\text{I}$  in 50  $\mu\text{L}$  of buffer different pH] at 40°C within 20 min.

the pH of the reaction medium was shifted toward alkaline pH, the radiochemical yield of  $^{125}\text{I}$ -Ceph decreased. This could be explained as increasing the pH value leads to a decrease in HOI which is responsible for electrophilic substitution reaction [14].

### 3.2. In Vitro Stability

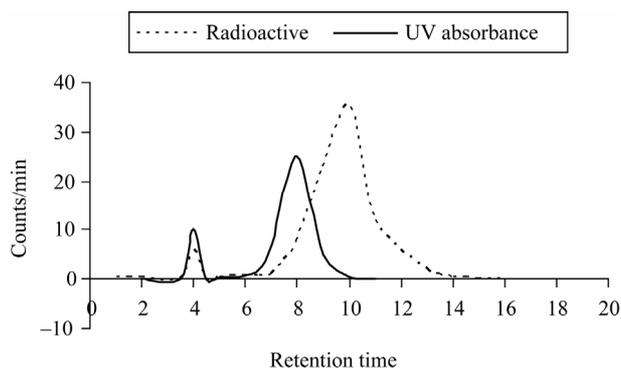
The stability of  $^{125}\text{I}$ -Ceph was studied in order to determine the suitable time for injection to avoid the formation of the undesired products that result from the radiolysis of the labeled compound. These undesired radioactive products may be toxic or accumulated in undesired organ. **Table 1** shows that  $^{125}\text{I}$ -Ceph is stable up to 24 h.

### 3.3. Radiochemical Purity

The free radioiodide was separated at retention time 4 min while  $^{125}\text{I}$ -Ceph is not completely separated from Ceph in **Figure 7**, which clarifies the overlapping between  $^{125}\text{I}$ -Ceph and Ceph, where the retention times are 10 and 8 min, respectively. The HPLC fractions were collected and evaporated under reduced pressure, dissolved in saline solution and sterilized by Millipore filter (0.22  $\mu\text{m}$ ) under aseptic conditions in a well type NaI (TI)

**Table 1.** *In vitro* stability of  $^{125}\text{I}$ -Ceph.

Time (h)	Radiochemical yield % of $^{125}\text{I}$ -Ceph
1	95 $\pm$ 1.5
2	96 $\pm$ 1.8
4	94 $\pm$ 2.1
8	93 $\pm$ 2.7
12	95 $\pm$ 1.9
24	97 $\pm$ 1.3



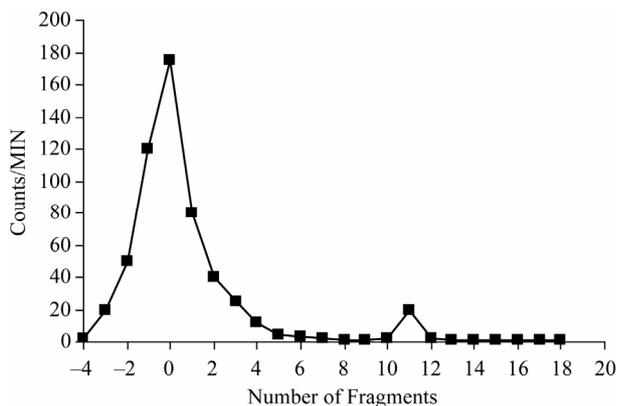
**Figure 7.** High-performance liquid chromatography elution profile of cephalexin, separated on reversed phase column (254\*4.6 mm at flow rate 1.5 mL/min).

detector connected to a single-channel analyzer [15].

The electrophoresis analysis of samples from the reaction mixture resulted in two peaks as shown in **Figure 8**, one corresponding to the free iodide which moved towards the anode with 11 cm distance while  $^{125}\text{I}$ -Cephalexin remained at the point of spotting.

### 3.4. Biodistribution

The  $^{125}\text{I}$ -Cephalexin was injected into normal mice via intravenous route to estimate its biological distribution. The data of this study is presented in **Table 2**. Data were collected and coded prior to analysis; all data were expressed as mean  $\pm$  SD. It is noticed that the blood uptake is high at 1/2 h equal to 24% post injection. This may be due to the binding of the drug with plasma protein. The uptake of the tracer by the liver is about 6.5% at 1/2 h post injection. This uptake slowly decreased to 5% at 4 h post injection. This low percentage uptake might be due to that the drug is not excreted from the liver. At 4 h post injection, the activity held by the collected urine increased to 20% due to the normal excretion of Cephalexin in urine, as 80% of cephalexin is excreted in the urine [16]. Also the data show that  $^{125}\text{I}$ -Cephalexin is held in the intestine by 8% and increased to 17% after 4 hr post injection. It was also absorbed by the stomach reaching 10% at 30 min post injection. This might be due to the



**Figure 8.** Electrophoretic pattern of  $^{125}\text{I}$ Cephalexin; condition: Solvent: 0.02 M phosphate buffer pH 7 paper type: cellulose acetate; 300 V; running time: 1.5 hours.

**Table 2.** Biodistribution of  $^{125}\text{I}$ -Ceph in normal mice.

Organs & body fluids	Detected dose/Organ percent at different intervals time post injection (min)			
	30	60	120	240
Blood	24 ± 1.4	20 ± 1.5	17 ± 1	11 ± 1.2
Bone	7.0 ± 0.6	6.8 ± 0.5	6.5 ± 0.2	6.0 ± 0.3
Muscle	15 ± 1.2	14 ± 1.2	11 ± 0.8	5 ± 0.5
Heart	0.4 ± 0.03	0.35 ± 0.02	0.3 ± 0.01	0.3 ± 0.01
Lungs	1.0 ± 0.05	0.9 ± 0.04	0.9 ± 0.03	0.8 ± 0.05
Liver	6.5 ± 0.5	6 ± 0.5	5.7 ± 0.6	5 ± 0.4
Kidneys	4 ± 0.3	3.5 ± 0.4	2 ± 0.2	1.8 ± 0.2
Urine	7 ± 0.7	10 ± 1.2	15 ± 1.1	20 ± 1.4
Intestine	8 ± 0.6	10.5 ± 0.7	15.8 ± 0.7	17 ± 0.9
Stomach	10 ± 1.0	16 ± 1.7	14.5 ± 1.1	12 ± 0.9
Thyroid	0.05 ± 0.1	0.08 ± 0.1	0.1 ± 0.07	0.15 ± 0.04

rapid absorption of the drug from the gastrointestinal tract [12]. The uptake of the muscle was 15% at 30 min post injection and decreased to reach only 5% at 4 h post injection.

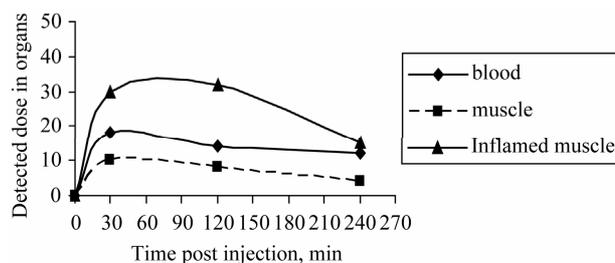
The biodistribution of  $^{125}\text{I}$ -Cephalexin tracer in *E. coli*-inflamed mice as cleared from the data presented in **Table 3**. Inflammation was induced by the injection of a suspension of *Escherichia coli* (*E. coli*) into the right thigh muscle and the left thigh muscle was taken as control. The uptake by the septic (inflamed) muscle was 30% at 30 min post injection and reached 15% at 4 h post injection, while the uptake of the non inflamed muscle (control) was 10% at 30 min post injection and decreased to 4% after 4 h post injection. Using the unpaired Student's test, statistical differences were assumed to be reproducible when  $p < 0.05$ , the difference between the

uptake of the inflamed muscle and the normal muscle was significant. This means that  $^{125}\text{I}$ -Ceph is able to localize in inflammatory foci induced by *E. coli*. The thyroid uptake was mostly ranged from 0.05 to 0.15% within 4h indicating that  $^{125}\text{I}$ -Cephalexin free form radioiodide and it is stable *in vivo*.

Furthermore, **Figure 9** summarizes the elimination of  $^{125}\text{I}$ -Ceph tracer from the blood and its washout from the normal muscle and the inflamed one (induced by *E. coli*). It was observed that at 30 min and 2 h post injection the uptake of the inflamed muscle was higher than the background, which is blood in our case, which gives us an indication that it is the best times for the imaging of the inflamed muscle to obtain a clear picture. While at 4 h post injection the ratio between the uptake of the blood and the inflamed muscle decrease which may affect the

**Table 3.** Biodistribution of  $^{125}\text{I}$ -Ceph in inflamed mice with *E. coli*.

Organs & body fluids	Detected dose/Organ percent at different intervals time post injection (min)		
	30 min	120 min	240 min
Blood	18 ± 1.2	14 ± 1.0	10 ± 0.7
Bone	5.6 ± 0.4	5 ± 0.3	5 ± 0.3
Muscle	10 ± 0.5	8 ± 0.6	4 ± 0.4
Inflamed Muscle	30 ± 1.2	32 ± 1.5	15 ± 0.8
Heart	0.4 ± 0.05	0.2 ± 0.03	0.2 ± 0.03
Lung	1 ± 0.1	1 ± 0.05	0.9 ± 0.1
Stomach	10 ± 1	11 ± 1.2	14 ± 1.5
Intestine	10 ± 0.6	14 ± 0.9	20 ± 1.2
Liver	4 ± 0.2	4 ± 0.3	5 ± 0.5
Kidneys	3 ± 0.2	2 ± 0.2	2 ± 0.09
Urine	6.5 ± 0.5	10 ± 1	24 ± 1.6
Spleen	0.3 ± 0.01	0.2 ± 0.01	0.2 ± 0.01
Thyroid	0.06 ± 0.02	0.09 ± 0.04	0.13 ± 0.1



**Figure 9.** Time course of blood elimination, normal muscle and inflamed muscle washout 30 - 240 minutes after the injection of  $^{125}\text{I}$ -Ceph into inflamed mice (induced with *E. coli*).

clarity of the imaging. The results also show that the uptake of the normal mice was lower than that of the blood along the different times. This will help us to differentiate easily between the normal and the inflamed muscle.

#### 4. Conclusion

The labeling of Cephalexine with radioactive iodine-125 was done. The optimum conditions of the labeling of Cephalexine to give a radiochemical yield of 95% were 200  $\mu\text{g}$  Cephalexine, 200  $\mu\text{l}$  of iodogen at pH 2 when the reaction mixture was heated at 40°C for 20 min. The ligand studied provides efficient labeling and good *in vitro* stability. All the obtained data demonstrate that the tracer was distributed rapidly throughout the body after intravenous injection and cleared through the urinary system. In addition, the biodistribution of the tracer in inflamed mice demonstrated the possibility of the use of this tracer as imaging agent for inflammatory foci.

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