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An investigation of the ^{125}I-radioiodination of colchicine for medical purposes

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A procedure for radioiodination of colchicine with iodine-125 is carried out via an electrophilic substitution reaction. The reaction parameters studied were colchicine concentration, pH of the reaction mixture, reaction time, temperature, different oxidizing agents and different organic media to optimize the conditions for the labeling of colchicine and to obtain a high radiochemical yield of the ^{125}I-colchicine (^{125}I-Col). Using 3.7 MBq of Na^{125}I, 1.25 mM of colchicine as substrate, 1.1 mM of chloramine-T (CAT) as oxidizing agent in ethanol at 60°C for 5 min, a maximum radiochemical yield of ^{125}I-Col (60%) was obtained. The specific activity of ^{125}I-Col obtained was 44.4 MBq/0.5 mmol, and the labeled compound was not completely separated and purified from Col by means of high-pressure liquid chromatography (HPLC), so the uncertainty in the purity may affect the distribution and clearance routes due to the expected competition between ^{125}I-Col and Col. The biological distribution in normal mice indicates the suitability of radioiodinated colchicine for imaging of muscles.

Keywords: colchicine; Na^{125}I; electrophilic substitution reaction; radioiodination

Introduction

Colchicine (col) is the main bioactive alkaloid of *colchicum autmnale* and is used in diagnosis and treatment of gout. It acts as a potent inhibitor of cellular mitosis by binding to microtubules. Colchicine, like many other cytotoxic drugs, enters the cell through the lipid bilayer by passive diffusion and binds reversibly to P-glycoprotein (Pgp).[1] Pgp is one of the mechanisms involved in multidrug resistance for chemotherapeutic drugs. Pgp-mediated transport of chemotherapeutic drugs has been studied using single photon emission computed tomography and positron emission tomography. [^{11}C]Colchicine has been reported to be a feasible substrate for imaging Pgp functions in tumors.[2,3] Colchicine derivatives were labeled by ^{99m}Tc for ^{99m}Tc–EC (ethylene dicysteine) colchicines,[4] by [^{99m}Tc$(CO)_3$ $(H_2O)_3]^+$ and by $[^{99m}Tc{\equiv}N]^{2+}$ for trimethylcolchicinic acid.[5] They were used in targeting tumors and reported to exhibit good tumor uptake.[4,5]

The present work deals with the labeling of colchicine by iodine -125 to study the factors, which affect the radiochemical yield. The labeled compound is purified by using HPLC for studying the biodistribution in normal mice.

The presumable structure for ^{125}I-Col via reaction of colchicine with Na^{125}I in the presence of CAT as oxidizing agent is shown in Scheme 1; where ^{125}I in the aromatic ring is in *ortho* position to methoxy group.

Experimental

Materials and methods

All chemicals used in the present work were of analytical grade. Colchicine was obtained from Adwic El-Nasser Pharmaceutical Chemicals Company, Egypt and was used without any purification. Absolute ethanol was used as a solvent without further purification. Double distilled water was used for all experiments. Chloramine-T [*N*-chloro-*p*-toluene sulfonamide salt (CAT)] from Aldrich and iodogen (1,3,4,6-tetrachloro-3α 6α-diphenyl glycoluril) from Pierce Chemical Company. Thin layer chromatography (TLC) aluminum sheets (20 × 25 cm) SG-60 F_{254} (Merck). Na^{125}I (185 MBq/5 μL) in diluted NaOH, pH 7–11 was purchased from Institute of Isotopes, Budapest, Hungary.

Equipment

Radioactivity was measured by means of a γ counter (Nucleus Model 2010) connected with a well type NaI (Tl) crystal. High-performance liquid chromatography (HPLC) [Shimadzu Model], LC- 9A pump, equipped with a Rheodyne injector (Syringe Loading Sample Injector-7125), UV Spectrophotometric Detector Shimadzu SPD-6A and stationary phase comprising a reversed phase nucleosil phenyl column (250 mm × 4.6 mm, 5 μm).

Labeling of colchicine with Na^{125}I

All experiments were carried out in a convenient round bottom flask (5 mL) with two necks. The flask was fitted with a reflux

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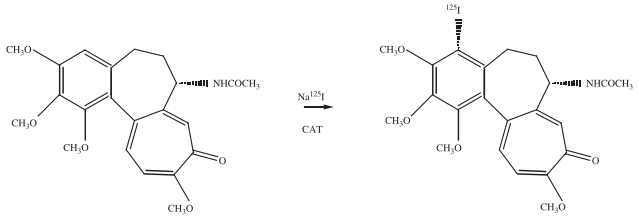

Scheme 1. The presumable structure of ^{125}I-CoI.

condenser on one neck and the other neck was fitted with rubber stopper for withdrawing samples. The flask was immersed in a thermostatically controlled water bath. 10 μL $Na^{125}I$ (3.7 MBq) was added in the bottom flask and they were dried by a vacuum line, then specific concentration of oxidizing agent, specific concentration of the substrate in a suitable organic medium were added. The reaction mixture was stirred with a magnetic stirrer and heated to specific temperature within suitable time. The different parameters that affect the radiochemical yield of ^{125}I-CoI like, colchicine concentration (50–300 μg), reaction temperature (25–100°C), type of oxidizing agent (CAT, iodogen), pH of the reaction mixture (2–11) and organic reaction medium (acetonitrile, methanol, ethanol, dimethyl sulphoxide (DMSO) or dimethyl formamide (DMF) were studied).

Radiochemical yield and purity

The radiochemical yield was determined by TLC and then radiochemical purity by HPLC.

TLC analysis

This technique was done using thin layer silica gel coated on aluminum sheet (20 cm × 20 cm). It was cut into strips each strip is 1 cm width and 13 cm length. The spotted point is placed 2 cm above the edge. The solvent used for developing was methylene chloride: ethyl acetate mixture (2:1 v/v). Radioiodide ^{125}I remained near the origin (Rf = 0–0.1), while the labeled compound of colchicine (^{125}I-CoI) moved with the solvent front (Rf = 0.8–1). The radiochemical yield was calculated by using TLC as follows:

$$\text{Radiochemical yield (\%)} = \frac{\text{Activity of labeled product} \times 100}{\text{Total activity}}$$

HPLC analysis

The radiochemical yield of ^{125}I-CoI was determined by direct injection of 5–10 μL, of the reaction mixture at the optimum conditions for obtaining the highest radiochemical yield, into HPLC with stationary phase comprising a reversed phase Nucleosil phenyl column (250 mm × 4.6 mm, 5 μm) using methanol: 0.05 M of ammonium acetate (48:52 v/v) as mobile phase with flow rate 1 mL/min. the labeled compound was collected by using a fraction collector and its activity was counted by using well type NaI (Tl) crystal connected with single channel analyzer. The free radioiodide was separated at retention time 5.5 min while the labeled compound (^{125}I-CoI) at 11.5 min.

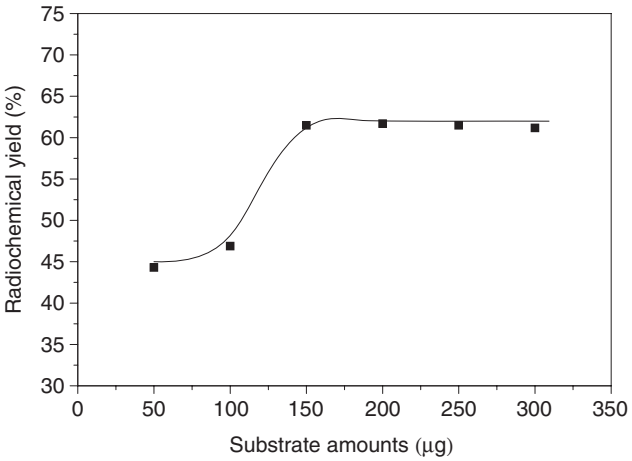

Figure 1. Variation of the radiochemical yield of ^{125}I-CoI as a function of CoI concentration [10 μL 3.7 MBq $Na^{125}I$ + x μg of CoI in ethanol + 100 μL 1.1 mM CAT] at 60°C within 5 min.

Results and discussion

Effect of colchicine concentration

The radiochemical yield of ^{125}I-CoI as a function of colchicine concentration was studied as shown in Figure 1. The results indicate that the radiochemical yield of ^{125}I-CoI increased from 44.3 to 60% by increasing the amount of colchicine from 50 to 300 μg. The radiochemical yield is not affected by the amount of colchicine higher than 200 μg. This may be attributed to the fact that the yield reaches the saturation value (60%) because the entire generated iodonium ions in the reaction are captured at that concentration of colchicine.

Effect of pH

Figure 2 shows the variation of the radiochemical yield of ^{125}I-CoI as a function of pH in presence of CAT as oxidizing agent. The results clarify that the pH is a significant factor that affects the labeling yield. The maximum yield was obtained at pH 7. This may be attributed to the fact that CAT works well as oxidizing agent to generate the iodonium ions around pH 7.5[6] and also due to the complete stability of colchicine at neutral solution, pH 7.[7] The good labeling yield of ^{125}I-CoI was obtained around that pH due to the protonation of the phenyl ring giving H^+, which was easily substituted by the radioactive iodonium ion I^+.[8] When the pH of the reaction medium was shifted toward the acidic region, the yield decreased to 25% at pH 2, this may be attributed to the predominance of ICl species, which have low oxidation potential less than HOCl species.[9] In case of alkaline region, the yield of ^{125}I-CoI is relatively poor, as a result of decreasing HOI, which is responsible for the electrophilic substitution reaction.[10]

Effect of oxidizing agents

Radioiodination of organic molecules has been performed by using a mild oxidizing agent such as CAT, which decomposes to hypochlorite anion that acts as an oxidizing agent, transforming iodine from I^- to oxidative state I^+. The influence of CAT concentration on the radiochemical yield of ^{125}I-CoI was firstly studied at pH 7 for 5 min to show that the labeling yield increased from 36 to 60% by increasing the concentration of CAT from 0.55

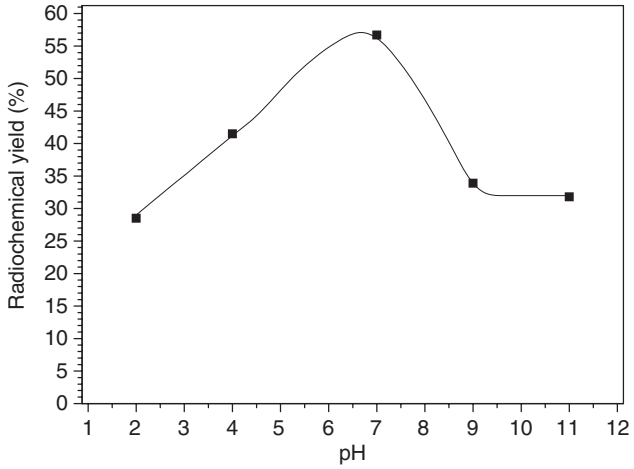

Figure 2. Variation of radiochemical yield of ^{125}I-Col as a function of pH [10 μL 3.7 MBq Na^{125}I+200 μg Col+100 μL buffer at different pH+100 μL 1.1 mM CAT] at 60°C within 5 min.

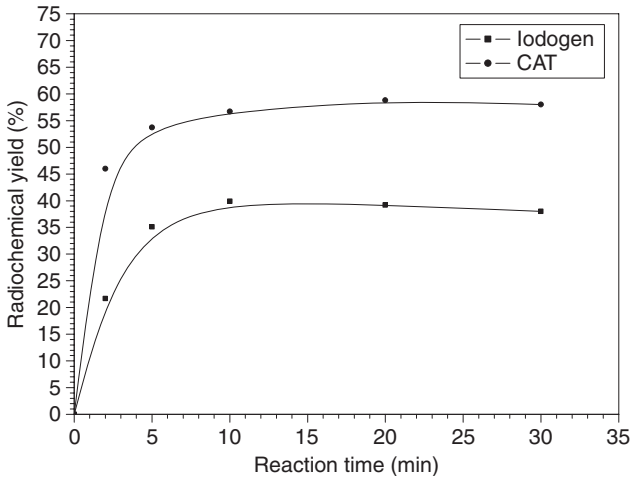

Figure 3. Variation of the radiochemical yield of ^{125}I-Col as a function of reaction time using different types of oxidizing agent [10 μL 3.7 MBq Na^{125}I+200 μg of Col +1.1 mM of CAT or 2.3 mM of iodogen] at 60°C.

to 1.1 mM. Increasing the CAT concentration above 1.1 mM leads to a decrease in the labeling yield due to the formation of undesirable oxidative side reactions like chlorination,[11,12] polymerization and denaturation of substrate.[10] The relationship between radiochemical yield of ^{125}I-Col and reaction time was studied at 1.1 and 2.3 mM for CAT and iodogen, respectively, as shown in Figure 3. The radiochemical yield of ^{125}I-Col increased rapidly to give 45% within 2 min and reached maximum maintained value 60% within 5 min in case of CAT, whereas the yield was 20% within 2 min in case of iodogen. The results confirm that CAT is a more effective oxidizing agent than iodogen. The lower radiochemical yield of ^{125}I-Col obtained by using iodogen as oxidizing agent, may be attributed to the insolubility of iodogen in water,[13] and so can be used as a thin film coating the wall of the radioiodination vessel, thus permitting labeling with very little contact of the organic compound with the oxidizing agent.[14,15]

Effect of reaction temperature

The radioiodination of colchicine by ^{125}I was carried out by studying the effect of reaction temperature (25–100°C) in

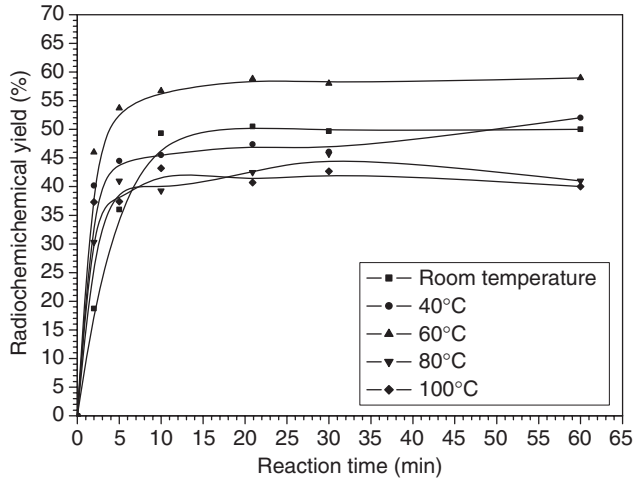

Figure 4. Variation of radiochemical yield of ^{125}I-col as a function of reaction time using at different temperatures [10 μL 3.7 MBq Na^{125}I+200 μg of Col +100 μL 1.1 mM CAT] at X°C.

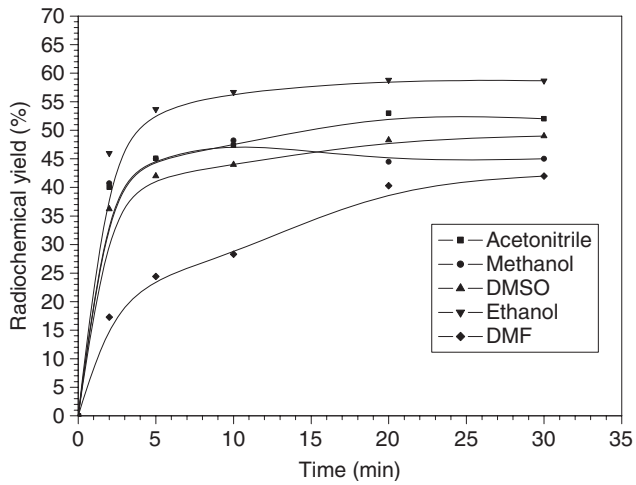

Figure 5. Variation of radiochemical yield of ^{125}I-Col as a function of reaction time using different organic solvents [10 μL 3.7 MBq Na^{125}I+200 μg of Col+100 μL 1.1 mM CAT] at 60°C.

presence of 1.1 mM CAT at pH 7 within 5 min as reaction time. The results are shown in Figure 4. The reaction temperature is a significant factor that affects the labeling yield, where the yield was very poor (20%) within 5 min at room temperature (25°C). By gradually increasing the reaction temperature from 40 to 60°C, the labeling yield increased from 45 to 55% within 5 min, respectively. By raising the temperature higher than 60°C to reach 100°C the labeling yield decreased to 35% within 5 min. The low yield of ^{125}I-Col at that temperature may be attributed to the thermal decomposition of the labeled compound or degradation of the oxidizing agent.[16]

Effect of solvents

The reaction between Colchicine (1.25 mM) and Na^{125}I (3.7 MBq) in the presence of CAT (1.1 mM) as oxidizing agent was examined in some organic solvents such as DMF, acetonitrile, DMSO, methanol or ethanol as shown in Figure 5. It is clear that within the first minutes a fast increase in the radiochemical yield of ^{125}I-Col takes place and reaches a saturation value of about 60% at 60°C within 10 min using ethanol as a solvent.

In spite of the advantageous characteristics of the dipolar aprotic solvents (DMF, DMSO), which include a high boiling point, ability to solvate a broad variety of solutes and to be useful in radioiodination reactions,[17,18] the labeling yield using DMF or DMSO as a solvent gave poor radiochemical yield of ^{125}I-Col.

In-vitro stability of ^{125}I-Col

The stability of ^{125}I-Col 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 might be accumulated in non-target organs. Table 1 clarifies the stability of ^{125}I-Col. The results show that ^{125}I-Col is stable up to 24 h.

Table 1. Stability of ^{125}I-Col

Time (h)	Percentage purity (%)
1	58.8
2	59.0
4	58.5
8	58.4
12	59.2
16	58.4
24	58.5

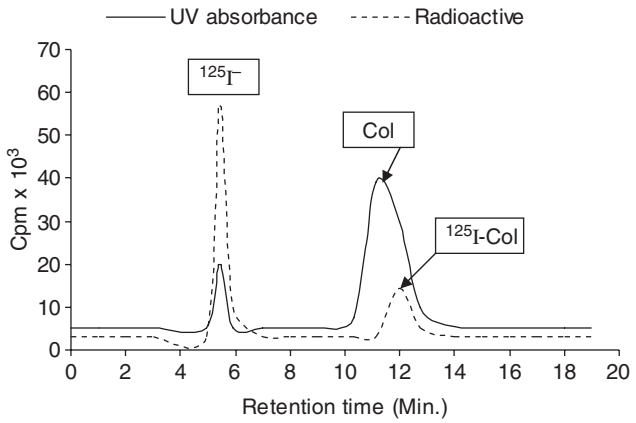

Figure 6. High-performance liquid chromatography elution profile of colchicine, separated on reversed phase column nucleosil (250 × 4.6 mm at flow rate 1 mL/min).

^{125}I–colchicine tracer

It was separated on semipreparative scale by means of HPLC as shown in Figure 6. ^{125}I-Col is not completely separated from Col in Figure 6, which clarifies the overlapping between ^{125}I-Col and Col, where the retention times are 12 and 11.5 min, respectively. The fractions were collected and evaporated under reduced pressure, dissolved in saline solution and sterilized by millipore filter (0.22 μm) under aseptic conditions,[19] to give specific activity 44.4 MBq/0.5 mmol of ^{125}I-Col.

Biodistribution study

In this study, the ^{125}I-Col was purified by HPLC, which was not completely separated from Col, so the distribution of ^{125}I-Col in normal mice was evaluated accompanied with Col as carrier. As a result of the fact that ^{125}I-Col is not purified from Col, there may be some competition between the ^{125}I-Col and Col for the binding sites and the uncertainty in the purity may affect the distribution and clearance routes. The experiment was carried out by injecting 3.7 MBq of ^{125}I-Col accompanied with Col into the tail vein of the albino mice. At each time point (0.5, 1 and 2 h), three mice were sacrificed to calculate standard deviation as shown in Table 2. ^{125}I-Col showed significant bone and muscle accumulation (11 and 22.3% ID/organ) within 120 min. There was also high uptake in stomach (8.4% within 120 min). The ^{125}I-Col was mainly excreted via the kidneys as 30% of the activity was detected in the urine at 120 min post injection.

Conclusion

Radioiodination of colchicine was carried out by electrophilic aromatic substitution at mild temperature for 5 min. This work has shown that CAT is useful for rapidly introducing radioiodine onto the aromatic ring in colchicine to obtain a maximum radiochemical yield of about 60% ^{125}I-Col. The labeled compound was purified by using HPLC for biological distribution, which clarifies the utility of radioiodinated cholchicine for imaging of muscles. ^{123}I-labeling of colchicine may therefore be of interest. A more efficient preparative purification system would be needed to ensure that the ^{123}I-iodocolchicine was free from colchicine.

Table 2. Biodistribution of ^{125}I-Col in normal mice

Organs and body fluids	Injected dose/organ percent at different intervals of time post injection (h)		
	0.5	1	2
Blood	25.6 ± 0.8	16.8 ± 0.4	9.3 ± 0.2
Lungs	1.4 ± 0.04	0.8 ± 0.02	0.8 ± 0.02
Heart	0.7 ± 0.02	0.3 ± 0.01	0.3 ± 0.01
Stomach	5.1 ± 0.2	13.7 ± 0.7	8.4 ± 0.4
Spleen	0.2 ± 0.01	0.1 ± 0.005	0.1 ± 0.005
Thyroid	0.9 ± 0.05	1.8 ± 0.1	2.1 ± 0.1
Muscle	12.1 ± 0.6	17.7 ± 0.9	22.3 ± 1.1
Liver	30 ± 1.5	15 ± 0.9	8 ± 0.4
Bone	2.7 ± 0.14	5.8 ± 0.3	11 ± 0.6
Intestine	4.6 ± 0.2	8.3 ± 0.4	4 ± 0.2
Kidneys	3 ± 0.2	1.7 ± 0.1	1.6 ± 0.1
Urine	3.6 ± 0.2	18.4 ± 0.9	30 ± 1.8

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