Labeling Study of Hydroxyethyl Starch with Tc^{99m} And Investigation of The Biokinetic Behavior In Experimental Animals

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Abstract

The labeling of stannous hydroxyethyl starch (Sn-HES) with technetium-99m (Tc^{99m}) has been studied.

It was found that the formation of Tc^{99m}-HES complex increased with raising of the pH, reaching a yield of over 90% at pH 6.5. Further experiments at pH 6.5 showed that the amount of reducing agent ($SnCl_2.2H_2O$) and the ligand (HES) has a little effect on the labeling yield of the complex.

The organ distribution in mice has shown that Tc^{99m}HES complex was cleared from the blood with relatively high accumulated in the liver and were no uptake of radiotracer in the lung, spleen and stomach.

This work demonstrates the conditions that are necessary for the labeling of HES with Tc^{99m} and its evaluation in experimental animals.

LABELING STUDY OF HYDROXYETHYL STARCH WITH TC^{99m} AND INVESTIGATION OF THE BIOKINETIC BEHAVIOUR IN EXPERIMENTAL ANIMALS

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دراسة تعليم مادة هيدروكسي أثيل النشا بالتكنيشيوم 99م وسلوكه البايولوجي في الحيوانات المختبرية

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الخلاصة

تم دراسة تعليم مادة هيدروكسي أثيل النشا HES بنظير التكنيشيوم 99م وقد وجد بان نسبة ناتج تكوين المعقد المعلم تزداد عند الدالة الحامضية 6.5 وان هناك تأثير قليل جدا لكلا العاملين المختزل وهو كلوريد القصديروز والمخلبي وهو هيدروكسي أثيل النشا على ناتج تعليم المعقد. أوضحت نتائج التوزيع البايولوجي في الحيوانات المختبرية (الفئران) بأن المعقد المعلم (T^{c99m}-HES) يخرج من الدم مع تجمع عالي نسبيا في الكبد وقليل في كل من الرئتين والطحال والمعدة. ان هذا البحث يوضح الظروف القياسية لتعليم مادة الهيدروكسي اثيل النشا وسلوكها البايولوجي في الحيوانات المختبرية.

Introduction

The main criterion in the selection of a proper ligand for labeling with technetium, in radiopharmacy, is to match the requirement of a radiopharmaceutical of a good biological specificity, where the desired target organ-to-background activity ratio is considerably high.

Technetium-99m radiopharmaceuticals are in widespread use owing to the availability and affordability of Mo^{99}/Tc^{99m} generators and the variety of kits for formulating the desired products. Together, they provide an array of specific tools for diagnosing a large number of diseases affecting the bones and major organs of the body such as the heart, brain, liver, kidney and thyroid. Nuclear medicine requires high quality radiopharmaceuticals and kits that are safe for administration and efficacious for a given application (1).

Technetium's labeled colloids are widely used for lymphoscintigraphy (2, 3). Colloidal tracers have two major limitation (a) its migration from the injection site is less than 35% in 24h (4) and (b) the uptake of the radioactivity by the nodes is dependent on the particle size and the functional state of the reticule-endothelial system (3,5). The difficulty in visualizing the lymphatic channel with radio colloids led some investigators to suggest a non colloidal non particulate tracer soluble in lymph fluid for visualization of lymphatic channels (6).

Tc^{99m}-Dextran has been evaluated by many authors in animals and human as lymphoscintigraphy agent and the result obtained were satisfactory (7, 6). An instant kit of stannous-dextran to be labeled with Tc-99m has been evaluated by Agha et. al (8).

Hydroxyl ethyl starch is a plasma substitute that in contrast to dextran causes no recognizable histamine release or allergic reaction in animals or humans (9). HES has a long intravascular half time (10), therefore, its

Tc^{99m} labeled derivative is expected to be cleared from interstitial tissues through the lymphatics and not to cross the blood capillaries. SADEK et al. have been reported on the labeling of hydroxyl ethyl starch with Tc^{99m} and its evaluation and comparison with other radiopharmaceuticals for visualization of lymphatic channels and lymph nodes in rabbits (11).

This work we demonstrate the conditions that are necessary for the labeling of hydroxyl ethyl starch with Tc-99m and its evolution in experimental animals.

Material and methods

All chemicals used in this study were of analytical grade obtained from commercial sources and used without further purification.

Hydroxyl ethyl starch (HES) was obtained from (Sigma chemical company, Poole, England). Stannous chloride dehydrate was obtained from (Riedel-De Haen, AG, Germany).

Sodium bicarbonate was obtained from (BDH chemicals Ltd, Poole, England). Whatman chromatograph paper (3MM) was obtained from (Whatman Ltd, Kent, England).

Tc^{99m}-Sn-HES was prepared by diluting of 5.0 ml of HES (4.0 mg/ml) by 5.0 ml of distilled water. The PH of HES solution was raised to 7.8-8.0 with 7% $NaHCO_3$ then the solution mixed with 0.1 ml of $SnCl_2.2H_2O$ (8.0 mg/ml). The initial PH of the preparation was measured and it was (4.0-4.5). The final PH was bringing to 6.5 by using few drops of 7% $NaHCO_3$. The final solution was passed through 0.2 μ Millipore filter membrane and aliquot of 2.0 ml mixed with 2.0 ml of TCO^-_4 eluate (8-15 mCi). The preparation was left to stand for 30 minutes at room temperature, and then radiochemical purity was determined with GCS and P.C. using acetone and 3MM paper.

Gel chromatograph column scanning (GCS)

Gel filtration using sephadex (AB, Pharmacia, Fine chemicals, Sweden) with column scanning technique was applied for determining the chemical state of Tc-99m in the radiopharmaceutical preparations (12).

A column with 1.5 cm i.d. was filled with swollen sephadex G-50-fine up to height of 30 cm and washed with 0.9% NaCl at PH 7.5. The sample (0.2-0.5 ml) to be analyzed was placed on the top of the column and eluted with 10 ml of 0.9% NaCl solution. The column was then sealed and scanned under a slit (1mm) collimated NaI (TI) crystal. The reduced hydrolyzed Tc^{99m} was found at a distance of 0.3 cm. at the top of the gel. The Tc^{99m}-pertechnetate and Tc^{99m}-HES complex were located at zones of 5-7 and 14-15 cm. below the top of the gel respectively.

Animal studies

Organ distribution studies following tail vein injection of 15-20μCi of labeled complex were performed in Swiss albino mice weighing 20-25 gm. The animals were killed at 5,15,30,60 and 120 min post injection by ether asphyxiation. The organs of interest and blood samples were taken from the dissected animals and counted in a well-type counter (Berthold – MAG 312, Germany). The total blood activity was calculated assuming that constitutes 7% of total body weight.

Biological studies

The organ distribution data of Tc^{99m}-HES preparation as a function of sacrifice time in mice are summarized in table (4) Tc^{99m}-HES was cleared from the blood and the relatively high accumulation in liver due to the uptake of HES molecules by this organ ,(13) There is no accumulation of radioactivity in the lungs, spleen and stomach.

The radioanalytical result together with biological finding, demonstrate the usefulness of the developed method for labeling of Sn-HES preparation with technetium-99m. Further studies are necessary to

evaluate the usefulness of the developed complex as lymphoscintigraph agent and comparison with other radiopharmaceuticals used for the same purpose.

Results and Discussion

Different radiochemical studies were performed using GCS method to optimize the parameters and condition necessary for the labeled of Sn-HES with Tc^{99m}.The effect of PH as determined by GCS technique is presented in table (1). It was found that the labeled efficiency increased as the PH raised from 3.0 to 6.5 reaching over 90% labeled yield. This finding gives a good agreement with the result obtained by SADEK et.al (12). Subsequent studies at PH 6.5 and a constant amount of the HES indicate that the stannous chloride has little effect on the formation of Tc^{99m}Sn-HES complex. The data in table (2) was obtained by GCS analysis showed that a wide range of stannous chloride (80-480 μg) can be used giving minor variation in the labeling yield. Table (3) shows the effect of HES concentration on the labeling yield of the complex. The result demonstrates that 4.0 mg per preparation is the optimum concentration to get high labeling yield. The results obtained from biodistribution experiments are shown in table (4).

Table (1) Effect of PH on the labeling yield of Tc^{99m}-HES as determined by GCS technique

PH	% of Tc^{99m} activity present as		
	Red-hydro-Tc^{99m} ±S.D	Free Tc^{99m} ± S.D	Tc^{99m}-HES ± S.D
3.0	17.1 ± 1.7	4.5 ± 0.4	77.6 ± 0.6
4.5	13.4 ± 0.9	3.8 ± 1.0	82.6 ± 0.7
6.5	2.6 ± 0.6	2.8 ± 1.1	94.6 ± 1.1
8.0	4.2 ± 1.2	4.6 ± 0.7	91.1 ± 0.7

Table (2) Effect of $Sncl_2.2H_2O$ concentration on the formation of Tc^{99m} HES as determined by GCS technique

$SnCl_2.2H_2O$ Conc. mg	% of Tc^{99m} Activity Present as		
	Red-hydro-Tc^{99m} ± S.D	Free Tc^{99m} ± S.D	Tc^{99m} –HES ± S.D
80	3.4±0.9	8.9±2.0	87±2.4
160	3.3±0.8	6.1±0.6	90.6±0.8
320	1.9±0.4	3.6±0.6	94.5±0.5
480	2.6±0.7	4.4±0.5	93.0±0.5

Table (3) Effect of HES concentration on the labeling yield of Tc-Sn-HES complex as determined by GCS technique

HES Conc. mg	% Of Tc^{99m} Activity		Present as
	Red-hydro-Tc^{99m} ± S.D	Free Tc^{99m} ± S.D	Tc^{99m} HES ± S.D
2.0	4.3±0.8	10.6±0.7	85.1±0.4
4.0	1.9±0.4	3.6±0.6	94.5±0.5
8.0	3.3±0.7	4.5±0.6	92.2±0.7
12.0	3.1±0.6	4.2±0.6	92.7±0.7
16.0	3.3±0.5	3.8±0.4	92.2±0.3

Table (4) Biodistribution of Tc^{99m}HES complex as a function of time in mice

Organs	% of administered activity ± S.D after				
	5 min.	15 min.	30 min.	60 min.	120 min.
Blood	13.2±0.7	7.4±0.8	6.1±1.2	4.2±0.1	2.5±0.3
Liver	16.6±0.7	14.4±0.8	11.2±1.1	9.6±0.8	8.9±0.8
Lungs	1.0±0.2	0.8±0.1	0.3±0.1	0.3±0.1	0.2±0.04
Spleen	0.5±0.1	0.4±0.1	0.2±0.04	0.2±0.04	0.2±0.06
Stomach	0.4±0.1	0.3±0.07	0.2±0.02	0.2±0.03	0.3±0.1
Intestine	5.8± 0.1	7.9±0.9	6.1±0.5	4.9±0.4	3.8±0.2
2 Kidneys	7.2±0.8	6.7±0.3	4.7±0.4	3.8±0.5	3.2±0.2

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