Pathophysiological consequences of acidaemia and alkalaemia on the biodistribution of a selected group of Technetium-99m radiopharmaceuticals.

بعض التغيرات الفسلجية المرضية الناتجة من تغير الأس الهيدروجيني للدم على التوزيع البايولوجي لبعض مجاميع المستحضرات الدوائية المعلمة بالتكنيشيوم -99م

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Abstract

Actively induced acidaemia and akalaemia have been achieved in male *Swiss albino* mice by the intraperitoneal injection of a known dose solutions (10mg\kg) of either NH_4Cl or $NaHCO_3$ solutions) for 5, 10 and 15 minutes, prior to administration of labeled agents (^{99m}Tc-Tin-colloid, ^{99mTc}-DTPA, and^{99m}Tc-HIDA). Applied for liver, kidney, and hepatobiliary system radiodiagnosis of treated animal were compared with results of biodistribution data of the control group. The results of biodistribution data of acidaemic animals (blood pH range 6.8-6.9) have shown no significant difference in the liver uptake when^{99m}Tc-Tin-colloid applied .Whereas, acidaemic animals have shown a significant($p<0.05$) in the renal uptake at 10 mints period ,when injected with ^{99m}Tc-DTPA. As well as these animals have shown a significant deferent ($p<0.05$) in the excretion behavior of ^{99m}Tc-HIDA from liver to intestine.

The results had shown a normal biological distribution of three scanning labeled agents in alkalaemic animal (blood pH range 7.7-7.8).

Keywords: ^{99m}Tc- Radiopharmaceuticals, effect of acidaemia and akalaemia on the biodistribution of ^{99m}Tc- Radiopharmaceuticals.

الخلاصة

أُستحدثت حالة التحمض والقلائية لدم ذكورا لفئران المختبرية من خلال الحقن داخل الغشاء البريتوني لجرعة محددة (10mg/kg) من مادة كلوريد الامونيوم أو بيكاربونات الصوديوم وتركت لفترات زمنية معينة (15,10,5دقيقة) قبل عملية الزرق الوريدي للمستحضرات الصيدلانية المعلمة بنظير التكنيشيوم-99م المستخدمة لإغراض التشخيص الطبي للكبد والكليتين والجهاز الصفراوي (^{99m}Tc-Tin colloid, ^{99m}Tc-DTPA, ^{99m}Tc- HIDA) على التوالي ، ومن ثم إجراء التوزيع البايولوجي لها ومقارنة نتائجه مع نتائج حيوانات السيطرة ،بينت النتائج إن حالة التحمض المستحدثة بواسطة كلوريد الامونيوم مدى الدالة الاسية 6.9-6.8) لم تؤدي إلى حدوث فرق معنوي في قابلية الكبد على التقاط القصدير المعلم بالتكنيشيوم-99م (^{99m}Tc-Tin-colloid) غير أن حقن المادة الحمضية (كلوريد الامونيوم) وخاصة الفترة 10 دقائق أدى إلى تأخير معنوي ($p<0.05$) في طرح ^{99m}Tc-DTPA، كما إن طرح ^{99m}Tc-HIDA من الكبد إلى الأمعاء تباطأ معنوي ($p<0.05$) عند حقن كلوريد الامونيوم لفترات مختلفة(15,10,5دقيقة) ،بينما لم يتأثر التوزيع البايولوجي للمستحضرات الصيدلانية المعلمة الثلاثة في حالة القلائية المستحدثة (معدل الدالة الأسية 7.7-7.8) في الفئران المختبرية.

Introduction

We could define acidaemia and akalaemia as departure plasma form the normal physiological range of pH .In acid-base disturbances the pH might reach extremely low values like 6.8 and could go up to as high as 7.8 such extremes are tolerated for brief period of time and could be sometimes lethal unless rapid compensation is set on .In chronic disorders of acid-base balance, the pH lies within the range of 7.2-7.5 would be compensated to the maximal possible extent. Both respiratory and renal mechanism is major involved in the compensation of the pH disturbance [1].

There are number of pathological conditions that are accompanied with metabolic acidosis such as severe diabetes, fasting, ingestion of acid and renal failure[2]. Similarly. Metabolic alkalosis arises in cases where absorbable antacids are ingested or as a consequence of the loss of hydrogen ion from gastric fluid or in association with hypocalcaemia from diuretic therapy [3].

Radiopharmaceuticals are used in the field of nuclear medicine as tracers in the diagnosis and treatment of many diseases. Many radiopharmaceuticals labeled with Technetium-99m (^{99m}Tc) which has many useful properties as a gamma-emitting tracer nuclide. Different radiopharmaceuticals based on technetium-99m used for imaging and functional studies of the brain , myocardium, thyroid, lungs, liver, gallbladder, kidneys, skeleton, blood and tumors [4,5] . In general, lipophilic compounds labeled with radio nuclides are used for liver imaging to evaluate the functional status of the hepatocytes and the potency of the biliary duct [6].Such as HIDA-hepatobiliray scan (liver function scanning agent). The use of radioisotopes and radiation is indispensable in the research of life science, especially in pharmaceutical sciences. In vitro and in vivo diagnostic researches in nuclear medicine[7].

Use radioisotopes in radiopharmaceuticals, many tracers were used like ^{99m}Tc, ^{181}Re, ^{131}I and others [8,9,10]. Also many compounds were subjected to be labeled with these radioisotopes like MAG3, MDP, DTPA and others [11,12,13]. Many items should be in consideration for radio-imaging, for example, radiopharmaceutical development for the kidneys must focus on achieving high-target selectivity and binding affinity, stability and slow metabolism in vivo and minimal nonspecific accumulation and urinary excretion [14].

The present study aimed to evaluate the consequences of the chemically induced abnormal value of plasma pH on the biological fate of three different scanning agents that were labeled with technetium-99m.

Materials and methods

1- Chemical materials: All chemicals used in this study were of analytical grade obtained from commercial sources and used without any further purification, NH_4Cl and $NaHCO_3$ from BDH chemical Ltd.pool-England, sodium pertechnetate was eluted from generator Amersham Plc England. The different diagnostic technetium-99m labeled scanning agents (^{99m}Tc- HIDA, ^{99m}Tc-DTPA, ^{99m}Tc-Tin-colloid) were in lyophilized form before labeling with technetium-99m pertechnetate.

Tin-hepatoscan (a liver scanning agent):-

Vial contents: 0.12 mg stannous florid.
0.965 mg Sodium florid.

DTPA-renoscan (kidney function scanning agent)

Vial contents: 10 mg Ca_3Na diethylene triamine pentaacetate.
0.36 mg stannous chloride.

Vial contents: 20mg N (2,6-dimethyl phenyl carbamoyl methyl)iminodiacetic acid.
0.45 mg stannous chloride dehydrates.

All of the above scanning agents were provided by Iraqi radiopharmacy research center.

2- Experimental animals: Two hundred ten male *Swiss albino* mice were used in biological distribution studies obtained from Iraqi biology research center.

3- Chemical induced of acidamia and alklamia: Acidamia and alkalaemia was induced by the intra peritoneal injection of 10mg/kg single dose of NH_4Cl and $NaHCO_3$[1,15].The former caused to lower the plasma pH to levels ranging between 6.8-6.9 for period of 5-15 minutes .while the latter caused to rise the pH value to range 0f 7.7-7.8 for similar period of time. Normal control values of blood pH were at 7.3-7.4.

4- Blood pH: For the purpose of studying the effects of acidaemia and akalaemia on deposition of Technetium-99m scanning agent were trialed the alteration in blood pH caused by the intraperitoneal doses of NH_4Cl and $NaHCO_3$ solution at three different periods of time (5, 10

and 15 minutes).Bleeding of the animal was carried out by making a cut under the arm and collecting a sample of oozing blood in small test tube.

5- **Biological distribution**: This method is routinely employed for testing the biological behavior of ^{99m}Tc- scanning agents. In our study, we determined the the biological fate of three kinds of scanning agents using the same method. The labeled sample preparation was injected into the tail vein of the test animal. The dose for each agent was 10-15 µCi/0.1 ml. The treated mice were left for 10 minutes in case of ^{99m}Tc-Tin-colloid, 5 minutes with ^{99m}Tc-HIDA and 3-5 minutes for^{99m}Tc-DTPA.The animals were sacrificed and organs of interest were removed and counted in well-scintillation counter(Gamma zint-Bf- 5300-Berthold FRG).The results obtained corrected to total administrated active dose.

6- Statistical analysis:
Data were statistically analyzed using SPSS statistical software. Level of significant was assessed by using the Analysis of Variance (ANOVA) test. The level of significance was shown using the Least Significant Difference (LSD) test. Values are given as mean $\pm$ standard error (mean ± S.E.) P values <0.05 were considered statistically significant.

Results and Discussion

Biodistribution of ^{99m}Tc-Tin-coloid in test animals(treated with NH_4Cl or $NaHCO_3$ for 5,10 and 15 minutes) was a minor change in the distribution of labeled agent in liver and other organs when compared to control values (table 1).One could notice that the percentage of labeled agent was increased in blood in the case of 5 and 10 minutes of treatment with $NaHCO_3$ but only a trivial alteration (75.2%) was observed in the percentage of labeled agent accumulation in the liver for 10 minutes when compared with the control value (80%), Therefore, we could comment that as far as the hepatic uptake of ^{99m}Tc-Tin-coloid was concerned, that is mean the kupffer cells
(responsible for engulfing colloids) [16] did not effected by alkalaemia and academia of the blood, so results shown no significant difference between alkalaemic and acidaemic test and control values.

The results of renal clearance of ^{99m}Tc-DTPA in test animals (treated with NH_4Cl or $NaHCO_3$ for 5,10 and 15 minutes) have noticed a prominent elevation in the percentage of labeled agent in kidneys (during 5-15 minutes NH_4Cl treated mice) when compared to control values (table 2).A significant difference ($p<0.001$) was determined statistically in case of 10 minutes treatment with NH_4Cl, while 5 and 15 minutes treatment yielded a non significant retention($p<0.2$ and $p<0.1$) of labeled agent in kidney respectively. On the other hand, the treatment with $NaHCO_3$ cased no observable difference in the biological distribution data in test and control animals.

The explanation of significant increase in the percentage of labeled agent (^{99m}Tc-DTPA) deposited in the kidney could be based on the nature of the renal compensation for acid-base imbalance created by excessive administration of NH_4Cl solution to animal by the intra peritoneal route , since the problem is primarily one of ridding the body of the acid ,the kidney would function by excreting the acid radicals through an ion exchange mechanism whereby the hydrogen ion is secreted in exchange for sodium .The acidic nature of ^{99m}Tc-DTPA would make it vulnerable to excreted rapidly by the kidney [17] , if it was not for the competitive blockade imposed the rate of excretion of hydrogen ions that was induced by the injected NH_4Cl solution .

Hepatobiliary excretion of ^{99m}Tc-HIDA in test animals (treated with NH_4Cl or $NaHCO_3$ for 5,10 and 15 minutes) was demonstrated in manner where biological distribution offered a good means of determining the extent of localization of labeled agent in regions of interest specially liver and intestine . The results of table 3 have shown a clear significant difference in the percentage values of labeled agent accumulated in liver and intestine of treated animals (NH_4Cl solution was injected intra peritoneal at 5 ,10 and 15 minutes prior to the administration of ^{99m}Tc-HIDA) in a comparison with the control values. This incidental influence of acidaemia causing delayed of the hepatic excretion of ^{99m}Tc-HIDA to the intestine which might be linked to the effect of this acid imbalance

on bilirubin with the plasma albumin[3] , thus leading to the displacement of bilirubin .The increase in the plasma concentration of unbound bilirubin would result in its enhanced uptake into tissues. Eventually, this could lead the competitive inhibition between the predominant bilirubin and ^{99m}Tc-HIDA, thus causing the significant delay in the excretion of the latter .This explanation could be strengthened by recalling the findings of other workers [18,19] in which they determined the interrelation-ship between the the bilirubin level and ^{99m}Tc-HIDA as a hepatobiliary radiotracer and their dependence on the carrier mediated organic anion transport mechanism . Result obtained has not shown any alteration in the normal excretion of ^{99m}Tc-HIDA from the liver to the intestine in experimental animals that were previously treated with $NaHCO_3$ for same period of time (5, 10 and 15 minutes).

Table 1: The biodistribution data of ^{99m}Tc-Tin-colloid in control and treated animals (for different periods) with intra peritoneal injection (10mg/kg) of either NH_4Cl or $NaHCO_3$.

Treated material	Period of treatment/min	% Blood	% Liver	% Lung	% Stomach	% Spleen	% Intestine	% Kidneys
NH_4Cl	5	0.33±0.03	87±2.7**	0.51±0.01	0.02±0.001	2.4±0.15	0.08±0.07	0.3±0.09
NH_4Cl	10	0.95±0.03	83.9±1.7**	0.3±0.01	0.02±0.001	2.6±0.002	0.30±0.001	0.3±0.002
NH_4Cl	15	0.32±0.01	77.9±2.5**	0.43±0.01	0.022±0.002	1.9±0.01	0.1±0.001	0.3±0.001
$NaHCO_3$	5	2.6±0.03	88.6±3.1	0.7±0.02	0.2±0.002	2.2±0.04	0.6±0.003	0.9±.001
$NaHCO_3$	10	3.4±0.11	77.2±2.1**	0.5±0.02	0.3±0.01	1.7±0.008	1±0.001	1.1±0.001
$NaHCO_3$	15	0.98±0.02	81.6±2.7**	0.51±0.01	0.2±0.001	1.3±0.001	0.4±0.002	0.4±0.001
Control	-	1.05±0.02	80±2.3	0.94±0.01	0.13±0.001	2.1±0.001	0.37±0.002	0.5±0.001

Values represent mean ± S.E. n=10

** No ,significantly different .

Table 2: The table show the biodistribution of ^{99m}Tc-DTPA in animals that were previous treated (for different period of time)with intra peritoneal injection (10mg/kg)of either NH_4Cl or $NaHCO_3$.

Treated material	Period of treatment/min	%Blood	%Liver	%Stomach	%Intestine	% Kidneys
NH_4Cl	5	10.96 ±1.03	3.7±0.01	0.4±0.001	3.7±0.03	10.5±0.07**
NH_4Cl	10	12.5±1.04	3.4±0.03	0.6±0.012	4.2±0.02	11.04±0.06*
NH_4Cl	15	10.5±1.06	3.0±0.033	0.4±0.002	3.25±0.02	7.5±0.33**
$NaHCO_3$	5	10.3±0.7	2.2±0.067	0.4±0.002	2.64±0.67	5.6±0.02
$NaHCO_3$	10	11.7±0.14	2.35±0.067	0.4±0.012	3.3±0.33	5.16±0.03
$NaHCO_3$	15	9.2±0.54	1.6±0.024	0.3±0.033	2.7±0.12	4.2±0.13
Control	-	11.97±0.12	2.45±0.021	0.3±0.012	3.7±0.03	6.0±0.67

Values represent mean ± S.E. n=10

*Significantly different ($p<0.05$).

** No, significantly different.

Table 3: The biodistribution data of ^{99m}Tc-HIDA in treated animals (for different periods) with intraperitoneal injection (10mg/kg) of either NH_4Cl or $NaHCO_3$.

Treated material	Period of treatment\min	%Blood	%Liver	%Stomach	%Intestine	%2 Kidney
NH_4Cl	5	2.9±0.01	39.6±2.06*	0.06±0.001	30.97±2.31*	3.6±1.12
NH_4Cl	10	3.4±0.02	37.0±1.12*	0.1±0.001	27.4±2.33*	3.98±0.33
NH_4Cl	15	3.8±0. 01	51.8±2.33*	0.03±0.001	28.8±1.67*	4.0±0.12
$NaHCO_3$	5	2.3±0.01	20.75±1.66	0.4±0.001	40.7±2.34	3.04±0.03
$NaHCO_3$	10	2.2±0.01	22.8±1.67	0.12±0.001	39.5±3.13	1.8±0.08
$NaHCO_3$	15	2.3±0.01	18.6±1.41	0.3±0.002	42.1±2.33	1.9±0.06
Control	-	2.83±0.01	23.9±1.33	0.4±0.001	41.25±1.67	3.2±0.03

Values represent mean ± S.E. n=10
* Significantly different ($p<0.05$).

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