The Use of Hexamine Cobalt(III) Tricarbonate Cobalt(III) as Oxidizing Agent in Glutathione Stability Test

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ABSTRACT

Hexamine cobalt (III) tricarbonatecobalt(III). $[Co(NH_3)_6.Co(C0_3)_3]$ had been used as oxidizing agent in glutathione stability test. A comparative study with acety1 phenyl hydrazine (ACP) was established. The average mean reduction of glutathione in red blood cells was (19.82 ±12.55) for G6PD normal people and (59.10 ±4.04) for G6PD deficient people when cobalt complex was used ,while the average mean reduction of glutathione in red blood cells was (11.90 ±8.75) for G6PD normal people and (34.99 ±11.70) for G6PD deficient people when (ACP) was used. It is possible to use the cobalt complex as a good substitute for (ACP) in the glutathione stability test.

Introduction

The glutathione stability test appeasr to be useful in diagnosing sensitive erythrocytes in vitro (1,2). Glutathione and glutathione stability test are useful and accurate tests to distinguish between glucose-6-phosphate dehydrogenase (G6PD) normal and deficient subject (3). It was found that glutathione stability test is reliable, accurate and reproducible for studying the effect of drugs and oxidant agents on (G6PD) deficient and normal subjects (3-6). Acetyl phenyl hydrazine (ACP) is not unique in its effect upon glutathione for primaquine , phenyl hydrazine, hydroxylamine, nitrofurantion , 1- and 2- naphthol and some naphthaquinone derivatives give similar results (7,8) Acetyl phenyl hydrazine happen to be a suitable oxidant for glutathione stability test which gives satisfactory results provided that adequate oxygenation occurs during the incubation.

In this work hexamine cobalt (III) tricarbonate cobalt (III) was used instead of (ACP) and a comparative study was conducted.

Methods

All chemicals used in this work were pure and of analytical grad.

1. Preparation of hexamine cobalt (III) tricarbonate cobalt (III) $[Co(NH_3)_6 . Co(Co_3)_3]$.

   This complex was prepared according to the method described by Bricker et al (9).

2. Glutathione stability test

This test was adopted to study the effect of hexamine cobalt (III) tricarbonatecobalt(III) and acetylphenylhydrazine (ACP) on red blood cell glutathione of normal and deficient erythrocytes and as follow : the following solutions were prepared

a) Standard SH group assay: in 100 ml volumetric flask, 100 mg of glutathione was placed and distilled

water was added to bring to final volume. Different concentrations (5, 10, 15, 20, 30, 40 and 50 mg/dl) were prepared from glutathione standard solution by dilution in order to establish a standard curve.

b) 5.5-dithio-bis-2-nitrobenzoic acid reagent (DTNB): In 100 ml volumetric flask 40mg of (DTNB) was placed and solution of sodium citrate (1 g/dl) was added to bring to final volume.

c) Phosphate solution: in 1000 ml volumetric flask 42.59 g of Na_2HPO_4 was placed and distilled water was added to bring to final volume.

d) Precipitating solution: In 100 ml volumetric flask 1.67 g of glacial metaphosphoric acid, 0.2 g ethylene diaminetetraacetic acid and 30g NaCl were placed and distilled water was added to bring to final volume.

Procedure

I) Blood samples were collected from volunteers from the outpatient clinics in Saddam Teaching Hospital in Basrah. Their ages ranged from 20 to 40 years. The following exclusion criteria were used in selecting volunteers.

Sicklers; thalassacemic subjects in haemolytic crisis: subjects received blood transfusion during the last one year and subjects taking oxidants. All parameters were measured within 24 hr.

II) In 10 ml test tube the following reagents were added: 0.2 ml of whole blood, 1.8 ml distilled water and 3 ml precipitating solution. The mixture was allowed to stand for 5 minutes then filtered. To the filtrate 8 ml of phosphate solution, 1 ml of (DTNB) were added and the absorbance was determined at 412 nm. The glutathione concentration was obtained from the following equation:

$$\text{GSH mg/dl of RBC} = \frac{G \cdot (f \quad)}{H}$$

III) In a test tube containing 5 mg (ACP) 1.0 ml of whole blood was placed and mixed well. In another test tube 1.0 ml of whole blood was placed then both test tubes were kept in the incubator at 37 °C for 1.0 hr. After incubation the GSH concentration was determined by procedure (II). The same steps was followed when 5 mg of cobalt complex was used.

Results

Figure No. 1 shows the standard curve used to obtain the value of GSH concentration. Table 1 shows the level of GSH and the percentage reduction in GSH concentration in the presence of cobalt complex and (ACP) for G6PD normal subjects. Table 2 shows the level of GSH and the percentages reduction in GSH concentration in the presence of cobalt complex and (ACP) for G6PD deficient people. Table 3 shows the statistical analysis of effect of cobalt complex and (ACP) on GSH level of G6PD normal and deficient people.

Discussion

From Table 3 the average mean reduction of glutathione in red blood cells was more when cobalt complex was used as oxidizing agent instead of ACP in both G6PD normal and deficient erythrocytes. The difference was not significant in the case of (G6PD) normal people while the difference was significant in (G6PD) deficient people. Oxidizing agent possess a strong affinity for electrons and cause other substance to be oxidized by abstracting electron from them (10). ACP have been used as oxidant in almost all the researches concerning the study of the effect of drugs on G6PD deficient people and ACP produce a marked reduction in red cells GSH level in G6PD deficient erythrocytes. But it was found in this work that cobalt complex give higher percentage of reduction in GSH level in G6PD deficient red blood cells.

Hexamine cobalt (III) has high redox potentials which if properly utilized could be useful in the determination of many compounds (II). G6PD deficient red blood cells were unable to maintain their GSH content when incubated with cobalt complex for 1.0 hr. Estimation of the GSH level in the erythrocytes incubated with cobalt complex may made it possible to identify primaquin sensitive persons accurately. It is clear that cobalt complex acts as a good oxidizing agent.

Table (1):The level of GSH and the percentage reduction in GSH concentration in the presence of Co complex and (ACP) for (G6PD) normal people.

Noof sample	GSH mg % alone	GSH level after incubation with 5mg ACP	% reduction	GSH level after incubation with 5mg cobalt complex	% reduction
1	53.33	40.00	24.99	30.00	43.74
2	137.83	121.26	11.76	102.70	25.48
3	38.46	37.17	3.35	35.89	6.68
4	79.48	69.23	12.89	67.94	14.51
5	83.33	80.55	3.33	72.22	13.33
6	55.26	42.10	23.8	39.47	28.57
7	81.57	78.94	3.22	76.31	6.44

Table (2):The level of GSH and the percentage reduction in GSH concentration in the presence of Cobalt complex and (ACP) for (G6PD) deficient people

No of sample	GSH mg % alone	GSH level after incubation with 5 mg ACP	% reduction	GSH level after incubation with 5mg cobalt complex	% reduction
1	46.66	26.66	42.86	20.00	57.13
2	47.40	30.10	36.49	20.10	57.59
3	46.52	33.20	57.59	18.10	61.09
4	52.47	39.30	25.10	25.20	51.97
5	55.31	35.21	36.34	20.30	63.29
6	48.70	36.20	25.66	28.10	57.70
7	46.24	36.55	20.95	16.20	64.96

Table (3):Statistical analysis of the effect of Cobalt complex and (ACP) on GSH level of G6PD normal and deficient people

No. of samples	% change mean ±S.D		Mean difference ±S.D	t value	Significant
14 G6PD normal	ACP	Cobalt	-7.92±5.782	-1.369	0.2 not. Sig.
	11.90 ±8.75	19.82 ±12.55			
14 G6PD Deficient	34.99 ±11.70	59.10 ±4.04	-24.10±4.67	5.15	<0.01 sig.

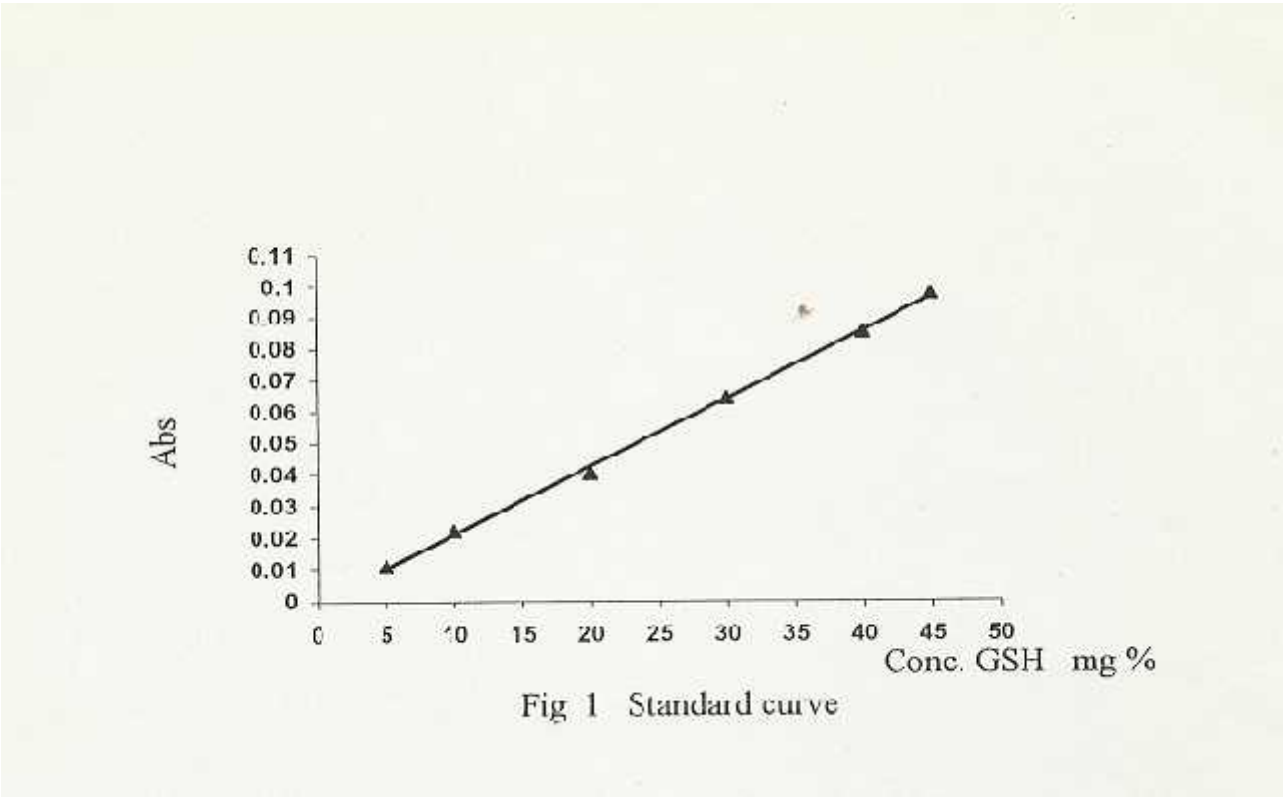

Fig 1 Standard curve

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استعمال هكسامين كوبلت تراي كاربونات الكوبت(III) في اختبار استقرارية الكلوتوثايون

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الخلاصه

تم في هذه الدراسة استخدام معقد الكوبالت سداسي امين كوبالت(III) ثلاثي كاربونات الكوبالت (III) $[Co(NH_3)_6.Co(Co_3)_3]$ كمادة مؤكسدة في كشف مستوى الكلوتاثايون في الدم.

اجريت دراسة لمقارنة هذه المادة مع مادة استايل فينيل هيدارزين (ACP). وكان معدل النسبة المئوية للخفض بالنسبة لمعقد الكوبالت هو (12.55 ± 19.82) للاشخاص غير المصابين بعوز نازعة الهيدروجين كلوكوز -6- فوسفات (G6PD) و (4.04 ± 59.10)للاشخاص المصابين بعوز نازعة الهيدروجين كلوكوز -6- فوسفات G6PD) فيما كان معدل النسبة المئوية للخفض لمادة (ACP) هو (8.75 ± 11.90) للاشخاص غي المصابين بعوزنازعةالهيدروجينكلوكوز -6- فوسفات(G6PD) و (34.49 ±11.70)للاشخاصالمصابينبعوزنازعةالهيدروجينكلوكوز-6- فوسفات(G6PD)عند اضافة كميات متساوية من كلا المادتين الى عينات الدم. يمكن استخدام هذا المعقد كبديل ناجح لمادة (APC).