Haemolytic potential of three chemotherapeutic agents and aspirin in glucose-6-phosphate dehydrogenase deficiency

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القدرة الكامنة على حلّ الدم لثلاث مركّبات كيميائية علاجية والأسبيرين في حالات عوز نازعة هدروجين الغلوكوز – 6 – فسفات

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خلاصة: تم اختبار التأثير المحتمل الحالّ للدم لثلاث مركّبات كيميائية علاجية وللأسبيرين، وذلك باستخدام اختبارات ثبات الغلوتاثيون في زجاج المختبر. فتم جمع الدم من أهالي البصرة بالعراق، حيث أظهرت دراسات سابقة زيادة انتشار عوز نازعة هدروجين الغلوكوز – 6 – فسفات. وتبيَّن أن البريماكين والكلورامفينيكول والسلفانيلاميد قد سبَّبت انخفاضات جوهرية مرتبطة بتركيزاتها، في مستويات الغلوتاثيون في كريات الدم الحمر المصابة بهذا العوز بالمقارنة بكريات الدم الحمر السوية. أما حمض الأسيتيل ساليسيليك فلم يكن له أي تأثير على مستوى الغلوتاثيون. وكان تأثر كريات الدم الحمر المصابة بالعوز متفقاً مع ما سبق نشره، ربما بسبب التماثل في أنماط توزُّع أشكال العَوَز المختلفة. ويقتضي الأمر إجراء دراسات على كل شكل محلي من أشكال العَوَز، مع دراسة مدى قدرة الأدوية الجديدة على حلّ الدم، قبل استعمالها في المناطق التي ينتشر فيها هذا العوز.

ABSTRACT The potential haemolytic effect of three chemotherapeutic drugs and aspirin was tested *in vitro* by gluthathione stability tests. Blood was collected from the local population of Basra, Iraq where previous studies had found a high frequency of glucose-6-phosphate dehydrogenase (G6PD) deficiency. Primaquine, chloramphenicol and sulfanilamide caused significant concentration-dependent reductions of glutathione levels in G6PD-deficient red cells when compared to normal red cells. Acetylsalicylic acid had no effect on glutathione level. The G6PD-deficient erythrocytes behaved as previously reported, probably due to similar patterns in the distribution of its variants. Studies on each local variant are warranted and new drugs should be tested for haemolytic potential prior to their introduction in areas where the deficiency is common.

Le potentiel hémolytique de trois médicaments chimiothérapiques et de l'aspirine dans l'anémie hémolytique enzymoprive (G-6-PD)

RESUME L'effet hémolytique potentiel de trois médicaments chimiothérapeutiques et de l'aspirine a été testé *in vitro* lors d'études de stabilité au glutathion. Des prélèvements de sang ont été effectués dans la population locale de Bassora (Iraq) où des études précédentes avaient trouvé une forte fréquence de l'anémie hémolytique enzymoprive (G 6 PD). La primaquine, le chloramphénicol et le sulfamide ont provoqué des réductions importantes dépendante de la concentration des niveaux de glutathion dans les globules rouges ayant un déficit en G-6-PD par comparaison avec les globules rouges normaux. L'acide acétylsalicylique n'a eu aucun effet sur le niveau de glutathion. Les érythrocytes ayant un déficit en G-6-PD se comportaient comme signalé auparavant, probablement en raison de caractéristiques similaires dans la répartition de leurs variantes. Les études réalisées sur chaque variante locale sont fondées et le potentiel hémolytique des nouveaux médicaments devrait être testé avant de les introduire dans des zones où ce genre d'anémie est courante.

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Introducton

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is one of the most common hereditary disorders. Millions of people worldwide are affected and there is a high frequency of the disorder in the malarious regions of the world [*1,2*]. There are variants of G6PD which are characterized by the level of enzyme activity and electrophoretic activity [*3,4*].

This deficiency in enzyme activity first came to attention following the observation that haemolytic anaemia developed in African Americans following the ingestion of primaquine [*5*]. G6PD-deficient erythrocytes develop haemolytic reactions usually following stressful conditions, the most common of which is exposure to drugs. Incubation of G6PD-deficient red cells with various oxidant drugs was shown to reduce glutathione levels in these cells. This reduction in glutathione was shown to be correlated with *in vivo* haemolytic effects and is predictive of such effects [*6–8*].

Different G6PD variants may influence the haemolytic effect of drugs. Subjects with unstable variants may be more sensitive than subjects with the Mediterranean variant, and the latter may be more susceptible than subjects with the A^- variant. This is due to differences in the level of G6PD activity in the red cells [*1,3*]. Other genetic differences in the red cells or variations in the rate of drug metabolism and the formation of various metabolites might influence the haemolytic effect of drugs [*3*].

G6PD deficiency is common in Basra where the frequency is 13% among adults and 11% among neonates [*9,10*]. A strong association between G6PD deficiency and neonatal jaundice and severe hyperbilirubinaemia has been also established [*11*]. A recent study of G6PD phenotypes in Basra found the distribution of variants of G6PD to be: B^+ (normal), 86.64%; Mediterranean, 8.06%; B^-, 4.9%; and A^+, 0.39% (Ajlan SK, Al-Naama LM, Al-Naama MM, unpublished data, 1998). This is comparable to adjacent areas [*4*]. This means that a large number of people may be at risk of haemolysis when they are prescribed certain potentially haemolytic drugs.

For the above-mentioned reasons we investigated the haemolytic potential of drugs commonly incriminated in G6PD-deficient red cells.

Subjects and methods

The subjects were divided into G6PD-normal and G6PD-deficient groups according to the fluorescent spot method as described by Beutler [*12*]. Also, G6PD enzyme activity was estimated by the rate of NADPH formation by measuring its absorbance at 340 nm according to the WHO method [*13*]. The enzyme activity was expressed as international units per gram of haemoglobin.

Blood samples were collected from patients attending outpatient clinics. G6PD-normal volunteers were recruited from hospital workers, medical students and their relatives. Blood specimens were drawn from G6PD-normal and G6PD-deficient subjects (10 samples each). Subjects with thalassaemia, sickle-cell anaemia and those with recent histories of blood transfusions were excluded. Blood was collected by venepuncture (8 ml) and transferred to heparinized tubes. The tubes were stored at 4 °C prior to measurement.

Glutathione level was determined by a method described by Beutler [*14*]. Glutathione stability was determined by incubation of blood with an oxidant substance

[6], the level was determined in duplicate and expressed as milligrams per decilitre. Reticulocyte count, haemoglobin and packed cell volume estimation were measured by standard laboratory procedures [15].

To determine the effect of various drugs, the oxidant was substituted with the drugs at various concentrations. The glutathione level was estimated before and after the mixture was incubated for 3 hours. Seven concentrations of primaquine were used (0.25, 0.5, 1.0, 1.5, 7.0, 14.0 and 18 µg/ml). For chloramphenicol, sulfanilamide and acetylsalicylic acid the following concentrations were studied respec- tively: 75, 150 and 225 µg/ml; 150, 300 and 450 µg/ml; and 300, 600 and 900 µg/ml.

Data were statistically analysed using the paired *t*-test to evaluate effects within each group and the unpaired *t*-test to evaluate effects between the groups. Correlation analysis was carried out to detect the dose-dependent effect.

Results

Table 1 gives results for the parameters measured in G6PD-normal and G6PD-deficient erythrocytes. The G6PD enzyme activity and the glutathione levels were statistically lower in deficient erythrocytes than they were in normal erythrocytes. No significant differences were detected in reticulocyte counts and packed cell volumes between the two groups.

Chloramphenicol produced a statistically significant reduction in glutathione both in G6PD-normal and G6PD-deficient erythrocytes (Table 2). The effect of chloramphenicol was greater in the deficient erythrocytes than in the normal erythrocytes ($P < 0.0001$). Sulfanilamide also produced a reduction in the glutathione level in both normal and deficient erythrocytes with greater effect on the deficient erythrocytes ($P < 0.0001$). Acetylsalicylic acid produced less effect in both the normal and the deficient erythrocytes than the above two drugs ($P < 0.02$). Primaquine produced a significant effect on both the G6PD-normal and G6PD-deficient erythrocytes, with greater effect on the deficient than the normal erythrocytes ($P < 0.0001$).

The effect of primaquine (Table 2) was dose-dependent with increasing effect as the dosage increased. Sulfanilamide had a similar effect. Chloramphenicol failed to

Table 1 **Values of parameters measured in G6PD-normal and G6PD-deficient erythrocytes**

Parameter	G6PD-normal (n = 40) Mean ± s	G6PD-deficient (n = 40) Mean ± s	t[a]	P-value
Enzyme activity (IU/g Hb)	6.55 ± 1.12	0.49 ± 0.77	21.72	< 0.0001
Glutathione (mg/dl RBC)	97.42 ± 13.2	64.10 ±13.1	11.23	< 0.0001
Reticulocytes	0.46 ± 0.19	0.64 ± 0.16	4.38	< 0.0001
Packed cell volume	0.36 ± 0.04	0.38 ± 0.05	1.98	< 0.05
Haemoglobin	13.1 ± 1.8	11.90 ± 1.9	2.9	< 0.0048

[a] *Unpaired* t-test *(two-tailed)*
s = *standard deviation* *RBC = red blood cells*

Table 2 **Effects of drugs on glutathione levels in G6PD-normal and G6PD-deficient erythrocytes**

Drug concentration (µg/ml)	G6PD-normal % change Mean ± *s*	G6PD-deficient % change Mean ± *s*	Difference normal–deficient %	*t*[a]	*P-value*
Chloramphenicol					
755	−5.0 ± 5.3	−16.3 ± 7.2	11.2	3.93	0.0005
150	−6.1 ± 4.6	−14.8 ± 6.9	8.9	3.38	0.005
225	−6.6 ± 4.5	−15.0 ± 7.3	8.5	3.12	0.005
Sulfanilamide					
150	−0.7 ± 2.1	−2.7 ± 5.8	1.90	1.03	0.3
300	−2.3 ± 3.9	−7.8 ± 6.5	2.29	2.29	0.025
450	−2.9 ± 2.9	−11.9 ± 6.9	3.93	3.93	0.01
Acetylsalicylic acid					
300	−3.9 ± 5.1	−3.5 ± 6.6	0.17	1.17	0.8
600	−5.2 ± 4.7	−5.1 ± 8.2	0.01	0.01	0.9
900	−9.0 ± 8.2	−6.3 ± 8.9	0.71	0.71	0.4
Primaquine					
0.25	1.5 ± 0.9	−6.2 ± 3.1	7.56	7.56	0.0005
0.5	0.8 ± 1.9	−16.5 ± 6.2	8.43	8.43	0.0005
1.0	0.7 ± 1.0	−25.3 ± 7.4	11.04	11.04	0.0005
1.5	0.1 ± 2.3	−43.0 ± 10.5	12.76	12.76	0.0005
7	−2.4 ± 4.2	−44.9 ± 7.3	16.10	16.10	0.0005
14	−8.4 ± 4.9	−56.1 ± 9.2	15.02	15.02	0.0005
28	−13.1 ± 4.2	−70.5 ± 12.1	14.14	14.14	0.0005

[a]*paired* t-*test*
s = *standard deviation*

show a dose-dependent effect on the G6PD-deficient erythrocytes.

The regression and correlation analysis of the drugs' effects on glutathione levels in G6PD-normal and G6PD-deficient erythrocytes are shown in Table 3.

Discussion

The development of the glutathione stability test [*6*] has provided a method for the identification of individuals whose red cells are susceptible to haemolysis by certain drugs. A positive glutathione stability test is characterized by a marked reduction in the glutathione levels of erythrocytes after incubation with the oxidant acetylphenylhydralazine, while in normal red cells the level is slightly decreased. It has been suggested that this test be used as one of the models to screen for possible haemolytic effect in G6PD-deficient subjects [*16*].

In this study, we found a marked reduction in glutathione level in G6PD-deficient red cells incubated with primaquine. Fewer effects were observed with chloramphenicol and sulfanilamide. However, no such effect was observed with acetylsalicylic acid.

It has been found that in the case of chloramphenicol there is a relationship be-

Table 3 **Glutathione levels (mg/dl) in G6PD-normal and G6PD-deficient cells incubated with and without drugs**

Drug concentration (µg/ml)	G6PD-normal % change Mean ± *s*	G6PD-deficient % change Mean ± *s*	*t*[a]	*P-value*
Chloramphenicol				
0	90.9 ± 14.9	59.1 ± 10.9	5.45	<0.0001
75	86.6 ± 16.7	49.8 ± 11.8	5.69	<0.0001
150	85.2 ± 13.5	50.6 ± 11.9	6.08	<0.0001
225	84.6 ± 12.5	50.4 ±10.8	6.10	<0.0001
r	0.98*	0.54		
Sulfanilamide				
0	96.4 ± 14.7	62.3 ± 18.5	4.56	0.0003
150	95.8 ± 15.4	58.6 ± 20.9	4.53	0.0003
300	94.3 ± 14.9	57.6 ± 18.9	4.56	0.0003
450	93.8 ± 16.1	54.9 ± 17.6	5.10	<0.0001
r	0.98*	0.99*		
Acetylsalicylic acid				
0	93.9 ± 16.3	69.3 ± 16.6	3.34	0.0036
300	90.5 ± 17.5	67.4 ± 18.2	2.89	0.0097
600	86.1 ± 17.9	66.2 ± 17.7	2.50	0.0220
900	85.3 ± 16.0	65.4 ± 18.4	2.50	0.0220
r	0.98*	0.97*		
Primaquine				
0	100.4 ± 9.6	63.3 ± 8.1	9.34	<0.0001
0.25	101.6 ± 7.8	59.7 ± 11.6	9.64	<0.0001
0.5	101.3 ± 7.6	51.3 ± 6.5	15.81	<0.0001
1	101.1 ± 7.0	54.9 ± 6.0	15.92	<0.0001
1.5	100.6 ± 7.1	34.8 ± 5.2	23.64	<0.0001
r	0.20	0.99*		
7	97.3 ± 12.1	36.1 ± 5.8	14.42	<0.0001
14	91.3 ± 11.5	27.3 ± 5.9	15.60	<0.0001
28	86.6 ± 10.5	20.0 ± 10.3	14.32	<0.0001
r	0.98*			

[a]*Paired* t-*test*
**Significant*
r = *correlation coefficient* s = *standard deviation*

tween the G6PD variant and the drug's haemolytic effect. Chloramphenicol causes haemolytic anaemia in G6PD-Mediterranean, while no such effect occurs in G6PD-Canton. The diseases associated with chloramphenicol usage may be responsible for the observed haemolytic effect [*17*]. Chloramphenicol caused a significant reduction in glutathione levels in our study, but as the effect was not dose-dependent, this needs further study.

Sulfonamides in therapeutic doses can cause acute haemolysis in G6PD-deficient subjects [*18*]. Beutler [*19*] found no reduction in reduced glutathione level with sulfanilamide in a concentration of 150 µg. In this study, no effect occurred at this concentration, but reduction did occur at the higher concentrations of 300 µg and 450 µg. It has been suggested that haemolysis due to sulfa drugs appears only when there is a low level of glutathione in the red blood cells or the metabolites of these drugs [*20*].

It has been reported [*16*] that the average level of glutathione does not decrease significantly in G6PD-deficient red cells compared to normal cells after incubation with 30 µg and 150 µg acetylsalicylic acid, and that, moreover, the usual therapeutic dose of salicylates does not cause haemolysis. However, the administration of high doses (100 mg/kg), as in the treatment of rheumatic fever, may produce severe haemolysis [*21*]. Some studies [*22*] have shown that acetylsalicylic acid produces haemolytic reaction in some G6PD-deficient subjects. This effect has been attributed to gentisic acid, a known aspirin metabolite with redox properties. It is obvious from our study that we cannot exclude the haemolytic effect of drug metabolites. In addition, the genetic polymorphism in drug metabolism may explain the *in vivo* variability in haemolytic response [*23*].

Primaquine is the prototype of drugs that cause haemolysis in G6PD-deficiency. Its haemolytic effect has been well documented in African American, Mediterranean and Asian varieties [*24,25*]. In this study, we found a dose-dependent reduction in glutathione level in G6PD-deficient erythrocytes incubated with primaquine; this is in agreement with other studies [*26,27*].

The observed haemolytic effect in this study does not differ greatly from that reported in the literature. This is probably due to the B^+ variant which predominates in the population of Basra (Ajlan SK, Al-Naama LM, Al-Naama MM, unpublished data, 1998). It would be interesting to examine the haemolytic effect of drugs upon different local variants. Testing of new drugs for haemolytic potential should be done prior to their introduction into areas with high frequencies of the G6PD-deficiency.

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Haemoglobin disorders and glucose-6-phosphate dehydrogenase deficiency are common in most countries of the Region and well organized services for these disorders are a priority. The Regional Office provided a wide range of health programmes to assist countries in prevention and control of genetic and congenital disorders and to increase awareness among health professionals and policy-makers regarding the size and magnitude of the problem in the Region.

Source: The Work of WHO in the Eastern Mediterranean Region. Annual Report of the Regional Director, 1 January–31 December 1998, *page 158. World Health Organization, Regional Office for the Eastern Mediterranean, Alexandria, 1999.*