

Effect of vitamin K1 on glucose-6-phosphate dehydrogenase deficient neonatal erythrocytes in vitro

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Abstract

Aim—To determine whether vitamin K1, which is routinely administered to neonates, could act as an exogenous oxidising agent and be partly responsible for haemolysis in glucose-6-phosphat-dehydrogenase (G-6-PD).

Methods—G-6-PD deficient (n=7) and control (n=10) umbilical cord blood red blood cells were incubated in vitro with a vitamin K1 preparation (Konakion). Two concentrations of Vitamin K1 were used, both higher than that of expected serum concentrations, following routine injection of 1 mg vitamin K1. Concentrations of reduced glutathione (GSH) and methaemoglobin, indicators of oxidative red blood cell damage, were determined before and after incubation, and the mean percentage change from baseline calculated.

Results—Values (mean (SD)) for GSH, at baseline, and after incubation with vitamin K1 at concentrations of 44 and 444 μM , respectively, and percentage change from baseline (mean (SD)) were $1.97 \pm 0.31 \mu\text{mol/g}$ haemoglobin, $1.89 \pm 0.44 \mu\text{mol/g}$ ($-4.3 \pm 13.1\%$), and $1.69 \pm 0.41 \mu\text{mol/g}$ ($-14.5 \pm 9.3\%$) for the G-6-PD deficient red blood cells, and $2.27 \pm 0.31 \mu\text{mol/g}$ haemoglobin, $2.09 \pm 0.56 \mu\text{mol/g}$ ($-7.2 \pm 23.2\%$), and $2.12 \pm 0.38 \mu\text{mol/g}$ ($-6.0 \pm 14.1\%$) for the control cells. For methaemoglobin (percentage of total haemoglobin), the corresponding values were $2.01 \pm 0.53\%$, $1.93 \pm 0.37\%$ ($-0.6 \pm 17.4\%$) and $2.06 \pm 0.43\%$ ($5.7 \pm 14.2\%$) for the G-6-PD deficient red blood cells, and $1.56 \pm 0.74\%$, $1.70 \pm 0.78\%$ ($12.7 \pm 21.9\%$), and $1.78 \pm 0.71\%$ ($20.6 \pm 26.8\%$) for the control red blood cells. None of the corresponding percentage changes from baseline was significantly different when G-6-PD deficient and control red blood cells were compared.

Conclusions—These findings suggest that G-6-PD deficient red blood cells are not at increased risk of oxidative damage from vitamin K1.

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Keywords: G-6-PD; methaemoglobin; vitamin K; haemolysis

Neonatal jaundice that is associated with glucose-6-phosphate dehydrogenase (G-6-PD)

deficiency may be the result of an acute haemolytic episode after exposure to identifiable chemical triggers.¹ However, this jaundice frequently occurs in the absence of any such exposure.² Using carboxyhaemoglobin (COHb) determinations corrected for inspired carbon monoxide as an accurate index of haemolysis and thus bilirubin production, these values were significantly increased in both non-jaundiced and jaundiced G-6-PD deficient neonates of Sephardic Jewish heritage, compared with non-jaundiced G-6-PD normal controls.³ The neonates had not been exposed to any known trigger of haemolysis. This haemolysis was probably the result of naturally occurring oxidants, but these babies may have been unintentionally exposed to a chemical trigger of haemolysis as part of their routine management. Routine administration of vitamin K1 to G-6-PD deficient neonates is not associated with severe haemolytic crises or hyperbilirubinaemia, but there is no information on whether the drug may precipitate a low grade haemolysis, as documented by the COHb data.³

This in vitro study aimed to determine whether vitamin K1 has an oxidant effect on G-6-PD deficient red blood cells which could be responsible, at least in part, for the increased haemolysis seen in affected neonates. The oxidant effect of vitamin K1 on G-6-PD deficient and control umbilical cord red blood cells was determined by measuring reduced glutathione (GSH) and methaemoglobin before and after incubation with a vitamin K1 preparation. Values for the former would be expected to decrease, and the latter to increase, following oxidative stress. An in vitro method was chosen, as withholding of vitamin K1 prophylaxis, which would have been a necessary component of a controlled clinical trial, may be associated with life threatening haemorrhage.

Methods

Umbilical cord blood from Sephardic term Jewish male neonates, at high risk for G-6-PD deficiency and born to Sephardic Jewish mothers at the Shaare Zedek Medical Centre, was collected into EDTA tubes after delivery of the placenta. The study samples were tested for G-6-PD deficiency using a qualitative colour reduction method (Kit No 400K, Sigma Diagnostics, St Louis, MO, USA). This kit accurately identifies G-6-PD deficiency in neonates.⁴ CPDA preservative was mixed with the blood in a ratio of 1 to 7. The blood samples were stored at 4°C for up to one week

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Table 1 Mean (SD) GSH and metHb values before and after incubation with vitamin K1

	Baseline	Incubation at 44 μ M vitamin K1	% change* from baseline	Incubation at 444 μ M vitamin K1	% change* from baseline
GSH (μ mol/g Hb)					
G-6-PD deficient (n=7)	1.97 (0.31)	1.89 (0.44)	-4.3 (13.1)	1.69 (0.41)	-14.5 (9.3)
G-6-PD normal (n=10)	2.27 (0.31)	2.09 (0.56)	-7.2 (23.2)	2.12 (0.38)	-6.0 (14.1)
Significance of % change			p=0.77		p=0.18
MetHb (% of total Hb)					
G-6-PD deficient (n=7)	2.01 (0.53)	1.93 (0.37)	-0.6 (17.4)	2.06 (0.43)	5.7 (14.2)
G-6-PD normal (n=10)	1.56 (0.74)	1.70 (0.78)	12.7 (21.9)	1.78 (0.71)	20.6 (26.8)
Significance of % change			p=0.2		p=0.2

*As the mean percentage change was calculated by first calculating the percentage change of each individual specimens, and then the mean percentage change of these individual percentages, values for percentage change in the table may appear unrelated to the mean measured values.

before being transferred to the Kaufmann Experimental Hematology Laboratory at Ben Gurion University of the Negev, Beer Sheva.

GSH and methaemoglobin concentrations in the umbilical cord blood samples were determined at baseline and the samples then divided into two. Each aliquot was thoroughly mixed with vitamin K1 (Konakion [phytom-enadione] Hoffman-La Roche, Basel, Switzerland) at concentrations calculated to be 44 μ M and 444 μ M, and then incubated at 37°C, for 60 minutes. These concentrations approximate to 10 and 100 times, respectively, the measured vitamin K1 concentrations found in neonatal serum after intramuscular injection of 1 mg.⁵⁻⁷ GSH concentrations were determined according to the method of Beutler⁸ by reducing dithiobisnitrobenzoic acid. Methaemoglobin production was determined spectrophotometrically, at 630 nm (Spectronic 2000, Bausch and Lomb, Inc., Rochester, NY, USA) according to the method of Winterbourn.⁹

Red blood cells from G-6-PD deficient neonates formed the study group, while those from G-6-PD normal infants comprised the control group. Mean (SD) values for GSH and methaemoglobin were calculated at baseline and after incubation with vitamin K1 at the two concentrations, respectively. Percentage differences between baseline values and those after the respective incubations with vitamin K1 were determined by first calculating the percentage difference for each individual sample and then the mean (SD) of these individual percentages. Comparisons of the percentage differences between the study and control groups were performed using Student's *t* test. Significance was defined as $p < 0.05$.

Results

Umbilical cord blood red blood cells from seven G-6-PD deficient and 10 control neonates were studied. Mean (SD) values for GSH and methaemoglobin at baseline, and after incubation with vitamin K1 at concentrations of 44 and 444 μ M/l, respectively, and the percentage difference from baseline values, are summarised in table 1. Mean percentage differences from baseline for study neonates were not significantly different from controls, for both reactions tested, and at both concentrations of vitamin K1.

Discussion

Bleeding due to vitamin K deficiency with the possibility of severe cerebral haemorrhage is a well known hazard of the neonatal period

(haemorrhagic disease of the newborn).¹⁰ Prophylaxis by administration of vitamin K immediately after birth was introduced in the 1950s and is recommended for all babies at birth.^{11 12} In Israel all neonates receive 1 mg of vitamin K1 immediately after delivery, in accordance with Ministry of Health regulations.

When vitamin K prophylaxis was first introduced, water soluble menadione (vitamin K3) was used. This vitamin preparation may be a strong oxidant. Administration of high doses of this drug, either to neonates or to pregnant mothers, was associated with the development of severe haemolysis, hyperbilirubinaemia, and kernicterus, especially in premature infants.¹³ G-6-PD deficient neonates were at greater risk for the development of these complications.^{14 15} Administration of the fat soluble vitamin K1 analogue was not associated with these complications¹⁶ and is now the recommended form of the vitamin. Using an *in vitro* method, Shahal *et al*¹⁷ studied the effect of vitamin K3 and K1 on G-6-PD normal neonatal erythrocytes. Vitamin K3, when incubated with erythrocytes, caused oxidative damage, as evidenced by the increased content of methaemoglobin and the depletion of GSH. In contrast, vitamin K1 did not cause these effects, either after incubation *in vitro*, or after therapeutic administration. Vitamin K1 has been administered safely to both G-6-PD normal and deficient neonates for many years, and has not been implicated as a cause of major haemolysis.

As G-6-PD deficient red blood cell antioxidant ability is even lower than the already decreased antioxidant ability of G-6-PD normal neonatal cells,¹⁸ we were concerned that vitamin K1 may possibly cause oxidative damage in G-6-PD deficient cells and thus be responsible for the low grade haemolysis documented in these neonates.³ It would have been interesting to have performed a controlled, *in vivo* study, of the effect of vitamin K1 administration to G-6-PD deficient neonates. However, as G-6-PD deficient newborns tolerate vitamin K1 prophylaxis without overt evidence of severe haemolysis, and as withholding of vitamin K1 may be associated with life threatening haemorrhage, we could not ethically justify delaying vitamin K1 administration for the purpose of a clinical study.

Several *in vitro* systems for testing the haemolytic effect of drugs on G-6-PD deficient red blood cells¹⁹ have been devised, in an attempt to imitate the *in vivo* situation. These

tests have some limitations, as in some instances injury to G-6-PD deficient red blood cells may be mediated not by the chemical compound administered, but rather by a metabolic product of that drug.¹⁹ Although some drugs can trigger haemolytic anemia in G-6-PD deficient individuals, they might not lyse red blood cells in vitro, but inflict oxidative injury instead. In the current in vitro study G-6-PD deficient and normal red blood cells were exposed to vitamin K1 at estimated concentrations higher than would be expected in neonatal serum following intramuscular vitamin K1 injection. Despite this, for both GSH and methaemoglobin values, and at the two concentrations of vitamin K1 used, the percentage change before and after incubation of the red blood cells was not significantly different between G-6-PD deficient cells and controls, and did not increase oxidative damage in the G-6-PD deficient cells.

The results of our study imply that vitamin K1 does not cause significantly greater oxidative damage in G-6-PD deficient neonatal red blood cells than in controls, and that the increased haemolysis found in G-6-PD deficient neonates cannot be attributed to routine administration of vitamin K1.

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