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Low Erythrocyte Glucose-6-Phosphate Dehydrogenase (G-6-PD) Activity and Susceptibility to Nitrite-Induced Methemoglobin Formation

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Considerable interest has recently focused on the identification and quantification of subsegments of the population that may be at increased risk to experience enhanced toxicity and/or carcinogenicity from environmental agents than the general public (CALABRESE 1979). In fact, the EPA has encorporated the knowledge of increased susceptibility in the development of criteria to support the derivation of ambient air and drinking water standards. For example, the ambient air standard for ozone recognizes the enhanced susceptibility of the asthmatics (CALABRESE 1978) while the drinking water standard for nitrate recognizes the enhanced susceptibility of neonates to develop methemoglobinemia (EPA 1979).

It is well known that persons with an erythrocyte G-6-PD deficiency have enhanced hemolytic susceptibility to numerous oxidant drugs and industrial chemicals. In addition, several recent theoretical studies have suggested that G-6-PD deficient humans may experience enhanced hemolytic risk following exposure to elevated levels of ambient ozone (CALABRESE et al. 1977) as well as several other environmental oxidant stressor agents (e.g. copper) (CALABRESE & MOORE 1979). That G-6-PD deficient individuals may be at such increased risk is of great social concern since 13% of the American Black male population have this deficiency (BEUTLER 1972). Since ethical considerations may preclude the in vivo experimental exposure of G-6-PD deficients to environmental oxidant stressor agents, it is important that an animal model be developed which accurately predicts the response of human G-6-PD deficient humans to oxidant stressor agents.

The intention of this paper is to evaluate the in vitro effects of sodium nitrite, a well known oxidizing agent and methemoglobin (MetHb) producer, on the responses of normal and G-6-PD deficient human erythrocytes as well as on the erythrocytes of Dorset sheep which also exhibit a similar enzyme activity as those humans with a G-6-PD deficiency.

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MATERIALS AND METHODS

Blood was collected in heparin from six G-6-PD deficient adult humans (4 negro males, A-variant; 1 homozygous negro female, A-variant; 1 male-variant Worcester, SNYDER et al. 1970); five young adult humans (3 males and 2 females) with normal G-6-PD activity; and six nonpregnant female sheep. Transported samples were maintained in ice packs until testing. All samples were tested within 3h of collection. Sampling occurred over a 6 week period. The blood sample from each subject was divided for control and test purposes. The testing procedure consisted of incubating 'deficient' and 'normal' blood with 2mM of sodium nitrite. Samples without sodium nitrite were incubated simultaneously under the same conditions for control purposes. The hematological parameters measured were selected primarily on the basis of their being widely accepted indicators of oxidative stress. The parameters included: methemoglobin (MetHb), levels of reduced glutathione (GSH). MetHb was measured according to the method by BROWN (1973) using potassium ferricyanide and potassium cyanide as reagents and measuring changes in optical density at 630 nm. A colorimetric reaction employing 5,5' -dithiobis nitrobenzoic acid (DTNB) was used to measure the amount of GSH in blood according to PRINS & LOOS (1969) at 421 nm. The measurement of G-6-PD activity was based on an ultraviolet kinetic enzyme assay kit packaged by Princeton Biomedix, Inc., Princeton, NJ 08540. Measurements were made with a spectrophotometer with a temperature controlled flow cell and automatic printer; calculator.

Data were computer analyzed by multiple variant analysis of variance (MANOVA) with the significance level set at 0.05. If MANOVA showed a difference in effect between blood samples, paired T-tests were performed to see the individual effects. However, the significance level was set at 0.01 for paired T-test to assure no loss of confidence in the final result.

RESULTS

Methemoglobin

Sodium nitrite treatment resulted in highly statistically significant increases in the MetHb levels of the blood of humans (normal and G-6-PD deficient) and sheep compared to their unexposed controls. Table 1 reveals that the sheep red blood cells were clearly the most sensitive while the normal human blood was the least sensitive. Further statistical analyses showed that the nitrite induced MetHb values were not statistically significantly different between normal and deficient humans but statistically significant ($p<0.05$) differences were found between sheep and human G-6-PD deficient blood.

TABLE 1

The Effects of the Nitrite Treatment on the Methemoglobin Formation (%)

Species	Treatment	Mean	S.D.	T-test
Sheep	Control	2.8	1.4	
	Nitrite	62.7	7.2	P<0.001
Normal Human	Control	0.9	0.9	
	Nitrite	29.3	7.7	P=0.001
G-6-PD Deficient Human	Control	1.1	1.3	
	Nitrite	37.7	7.1	P<0.001

Glutathione (GSH)

Although sodium nitrite is a potent MetHb producer, no statistically significant changes were observed in the GSH content as a result of the sodium nitrite treatment (Table 2). Both the sheep and the normal humans had a slightly but insignificantly increased GSH content after the exposure to sodium nitrite. Similarly, sodium nitrite slightly but insignificantly decreased the GSH content of the G-6-PD deficient humans.

TABLE 2

The Effects of the Nitrite Treatment on the GSH Contents (mg %)

Species	Treatment	Mean	S.D.	T-test
Sheep	Control	69.7	9.5	NS
	Nitrite	72.1	8.5	
Normal Human	Control	75.0	17.2	NS
	Nitrite	78.9	19.1	
G-6-PD Deficient Human	Control	47.0	7.0	
	Nitrite	42.7	6.7	NS

'NS' indicates a 'P' value of > 0.05.

G-6-PD Activity

Sodium nitrite treatment caused small absolute changes in the erythrocyte G-6-PD activity of the three experimental groups with insignificant increases occurring in the sheep and normal humans while a significant ($p<0.05$) decrease of from 1.63 to 0.97 IU occurred in human G-6-PD deficient blood.

DISCUSSION

It has been shown that sodium nitrite is a more effective MetHb producer in red cells which have a low level of G-6-PD activity than in normal human red cells with the sheep erythrocyte being particularly sensitive. These results are generally consistent with an earlier report by BREWER et al. (1962) which demonstrated that the administration of sodium nitrite to G-6-PD deficient and normal humans resulted in higher levels of MetHb in the deficients than normals. As the daily dosage of sodium nitrite increased from 1 to 4 gm, there was a marked increase in sensitivity of the G-6-PD deficient red cells to develop increased levels of MetHb.

In light of the fact that G-6-PD deficiency is extremely widespread in the U.S. Black male population and that sodium nitrite is widely used as a food preservative, further research should be designed to more comprehensively evaluate the extent to which G-6-PD deficient persons may be at increased risk to nitrite induced MetHb formation.

Finally, since the red cells of sheep (Table 1) and human G-6-PD deficients (BREWER et al. 1962) have enhanced susceptibility to nitrite induced MetHb formation, it is suggested that further research concerning the possible use of sheep red cells as a model for predicting the response of human deficient G-6-PD red blood cells to oxidant stressor agents is warranted.

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