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Low Glucose-6-Phosphate Dehydrogenase Activity and Increased Sensitivity to Paraquat Toxicity

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Considerable interest has recently been directed toward identifying the possible adverse human health effects of the herbicide paraquat. This is because the Mexican government since late in 1975 initiated the spraying of illegally grown marijuana fields with paraquat. If the plants are harvested before they are destroyed by the herbicide treatment, the paraquat will persist on dried leaves. About 60% of marijuana used in the U.S. originates in Mexico and about 20% of these have been reported to be contaminated to varying degrees with paraquat but with a mean level of 87 ppm. When marijuana is smoked, 60-70% of the paraquat present is coverted to bipyridine, a compound with similar toxicity as paraquat. If a person smoked 1 g of marijuana per day containing 87 ppm paraquat, an individual may inhale up to 0.1 µg of paraquat and 226 µg of bipyridine (WHALEN 1978).

Animal experiments have shown that most deaths from paraquat poisoning result from injury to the lung, an organ with exceptionally high tissue oxygen tension (FISHER et al. 1973). The lungs of rats dying from paraquat poisoning appear the same as those that die from oxygen poisoning. In fact, oxygen and paraquat have synergistic effects in that rats exposed to both paraquat and oxygen die sooner than from either agent alone. It has also been reported that there is an enhanced retention of paraquat in lung relative to other organs (FISHER et al. 1973). Moreover, BUS et al. (1976) reported that in vitro mouse lung microsomes catalyzed an NADPH-dependent, single electron reduction of paraquat. Therefore they suggested that lung tissue was capable of reducing paraquat to the free-radical form. ROSE et al. (1976) indicated that paraquat increased the oxidation of NADPH and stimulated the pentose-phosphate pathway. AYER & TIERNEY (1971) have shown that young rats are more resistant than older rats to pulmonary injury from oxygen and from paraquat, and that this resistance parallels the activity of the hexose monophosphate pathway, a major source of NADPH. BUS et al. (1976) also found that paraquat toxicity was markedly increased by deficiences of the anti-oxidants selenium and vitamin E. Furthermore, they reported that rats chronically exposed to 100 ppm paraquat in the water had elevated pulmonary activities of G-6-PD and GSH reductase, two enzymes of the pentose phospate pathway. From these studies BUS et al. (1976) speculated that three possible mechanisms may be involved in the detoxication of paraquat: 1) scavanging of superoxide radicals by superoxide dismutase; 2) antioxidant effects of vitamin E in terminating free

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radical chain reactions of paraquat induced lipid peroxidation and 3) the stimulation of GSH peroxidase system enzymes (i.e. GSH peroxidase, GSH reductase and G-6-PD).

In light of the proposed adaptive response to paraquat exposure of enhanced activity of GSH peroxidase system enzymes, it is proposed that individuals with a G-6-PD deficiency should be at increased risk to the oxidative damage caused by paraquat toxicity. Since 13% of the American black male population is known to experience a deficiency of erythrocyte G-6-PD, the issue of whether such a deficiency enhances paraquat toxicity is of extreme social importance. The intention of this paper is to evaluate via an in vitro study whether paraquat toxicity may be inversely related to the activity of erythrocyte G-6-PD in human deficients and in sheep (Dorset strain) which also have similar enzyme activity as those humans with a G-6-PD deficiency.

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 participant was divided for control and test purposes. The testing procedure consisted of incubating 'deficient' and 'normal' blood with 2mM of copper acetate at 37C in a water bath for two hours. The possible effect of the acetate ion on the blood characteristics studied was controlled by using sodium acetate at the same concentration. Samples without copper acetate and sodium acetate 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 (MetH), levels of reduced glutathione (GSH), red cell acetylcholinesterase activity (ACHase), and G-6-PD. MetH was measured according to the method by BROWN (1973) using potassium ferricyanide and potassium cyanide as reagents and measuring change in optical density at 630nm. 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 421nm. 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. Determination of red cell ACHase was according to ELLMAN et al. (1961). Changes in ACHase were evaluated since this enzyme is known to be intimately associated with the red cell membrane. GOLDSTEIN et al. (1968) have stated that decreased levels of ACHase activity implied that the red cell membrane has been altered or disrupted in some manner.

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-tests to assure no loss of confidence in the final result.

RESULTS

Methemoglobin. Paraquat treatment was found to increase the MetH levels in both sheep ($p<0.01$) and G-6-PD deficient humans ($p=0.1$) while not affecting normal humans (Table 1). While the absolute increase in MetH levels was rather modest especially with respect to the G-6-PD deficient human blood, both the sheep and human deficient blood exhibited approximately 300% increases in MetH when compared to their unexposed controls.

TABLE 1

The Effects of the Paraquat on the Methemoglobin Formation (%)

Species	Treatment	Mean	S.D.	T-test*
Sheep	Control	2.7	1.4	p=0.01
	Paraquat	8.4	3.0	
Normal Human	Control	0.9	0.8	NS
	Paraquat	0.4	0.9	
G-6-PD Deficient Human	Control	0.8	1.1	NS
	Paraquat	2.5	2.1	(p=0.1)

*NS indicates a 'p' value of > 0.05.

Glutathione (GSH). Reduced glutathione levels in both sheep and G-6-PD deficient human blood were decreased by about 11% ($p=0.002$) and 15% ($p=0.1$), respectively, by paraquat treatment while the GSH levels of normal human blood remained essentially unchanged (Table 2). Paraquat treatment resulted in no statistically significant changes in the G-6-PD activity in any of the blood groups tested.

TABLE 2

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

Species	Treatment	Mean	S.D.	T-test
Sheep	Control	69.6	9.52	p=.002
	Paraquat	61.6	9.93	
	% change	-11.5%		
Normal Human	Control	75.0	17.18	NS
	Paraquat	74.7	14.93	
	% change	- 0.3%		
G-6-PD Deficient Human	Control	47.0	7.03	NS
	Paraquat	39.9	8.83	(p=0.1)
	% change	-15.0%		

DISCUSSION

It has been demonstrated that human G-6-PD deficient and especially sheep blood are more sensitive to the oxidative stress of paraquat as measured by increases in MetH levels and decreases in GSH. These findings are consistent with the predictions of BUS et al. (1976) that paraquat may be detoxified in part via the increased activity of GSH peroxidase enzymes including G-6-PD. However, the sensitivity of these low G-6-PD red blood cells to paraquat is relatively modest as compared to similar studies with other oxidant stressors such as copper, nitrite, and chlorite in which similar concentrations of these agents cause more pronounced changes in either MetH and/or GSH levels than did the paraquat (HO 1979). It is not known what incubation of red cells with 2mM paraquat is in gram equivalents of marijuana smoked assuming a contamination level of 87 ppm paraquat because of the lack of knowledge concerning tissue distribution, metabolism and detoxification rates of paraquat. However, these *in vitro* findings suggest that further research is warranted in order to more precisely evaluate the possible increased susceptibility of human G-6-PD deficient individuals to paraquat. In addition, the similarity in sensitivity of sheep and human G-6-PD deficient red blood cells to paraquat induced oxidant stress suggest that sheep (Dorset strain) is worth pursuing as a potential model to predict the responses of human G-6-PD deficient red cells at least *in vitro* and that sheep as farm animals may be at increased risk to the toxicity of paraquat if their fodder contains significant residual paraquat.

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