TOLERABILITY OF IBUPROFEN AND FLURBIPROFEN IN G-6-PD DEFICIENT SUBJECTS: *IN VITRO* STUDY

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1 A 'GSH (reduced glutathione) stability test' which consists of incubating blood samples with acetylphenylhydrazine and other test drugs and measuring GSH level before and after incubation, was carried out.

2 Application of this test to the blood of eight normal volunteers and twelve known G-6-PD deficient persons demonstrated that acetylphenylhydrazine and primaquine produced a significant decrease in GSH levels in G-6-PD deficient red cells compared to the reduction seen in normal red cells incubated with these drugs. No such change was observed with aspirin, ibuprofen and flurbiprofen.

3 The results of this *in vitro* study seem to indicate that ibuprofen and flurbiprofen are relatively innocuous in G-6-PD deficient individuals.

Introduction

A number of drugs are known to produce haemolysis of red cells on administration to G-6-PD deficient persons (Beutler, 1969). Acetylsalicylic acid, a widely used analgesic and anti-inflammatory agent and some other analgesic drugs are also reported to produce haemolysis of G-6-PD deficient red cells (Beutler, 1969). Hence, selection of drugs becomes a problem for this group of patients.

G-6-PD deficiency is a very widespread disorder, affecting perhaps 100 million individuals in the world, although some populations are more affected than others (WHO, 1967). The incidence of this deficiency is reported to be as high as 16–53% in certain communities (Goldstein, Aronow & Kalman, 1969), which is significant enough to merit investigation of tolerability to commonly used non-steroidal anti-inflammatory agents.

An *in vitro* test, glutathione stability test, has been reported to be useful in detecting sensitivity of red cells deficient in glucose-6-phosphate dehydrogenase to drugs (Beutler, 1957; Baxi, Soni & Vaidya, 1978).

Therefore, we decided to undertake an *in vitro* study to detect the effect of two non-steroidal anti-inflammatory drugs (NSAID), ibuprofen and flurbiprofen on G-6-PD deficient red cells.

Method

Human blood specimens, drawn fresh from eight

healthy volunteers and twelve known G-6-PD deficient persons with heparin as the anticoagulant, were used in the study. All G-6-PD deficient persons were Indian Parsees. High incidence of G-6-PD deficiency has been reported in Indian Parsees (Baxi, Balkrishnan, Undevia & Sanghvi, 1963). All the healthy volunteers were males aged between 20–40 years. G-6-PD deficient persons included two male children aged 11 years and ten adults (eight male and two female) aged between 25–70 years. None of the G-6-PD deficient persons were clinically symptomatic. However, they were aware of their G-6-PD deficiency before selection for the study.

Two G-6-PD deficient persons (one adult male and one adult female) gave a past history of marked haemolytic reaction to some sulphonamide preparations. All of them, however, were careful in selecting drugs for their ailments. None of these donors had received any drug for a week prior to collection of blood samples. Blood (20 ml) was collected from each donor for the study.

Preparation of tubes containing test drugs: Solutions of drugs were prepared and dispensed in 25 ml Erlenmeyer conical flasks. Appropriate quantities of drug solutions were taken in test tubes and dried in the oven and stored in dark until use. The drugs and their concentrations used is shown in Table 1. Two concentrations of each test drug— aspirin, ibuprofen and flurbiprofen were selected in order to cover the concentrations, one which is likely to be achieved in

Table 1 Concentrations of drugs used

Drug	Solvent	Concentration/ ml of blood
Acetylphenylhydrazine	Acetone	5 mg
Primaquine	Water	6 mg
Flurbiprofen	Alcohol	12 µg
Flurbiprofen	Alcohol	60 µg
Ibuprofen	Alcohol	20 µg
Ibuprofen	Alcohol	100 µg
Aspirin	Water	30 µg
Aspirin	Water	150 µg

the serum after therapeutic doses and the other five times higher than this.

Reduced glutathione determination: Reduced glutathione (GSH) was estimated by DTNB method of Beutler, Duron & Kelly, (1965). GSH contents were expressed as mg/100 ml red cells.

The 'GSH stability test': The procedure described below was employed to test the stability of red cells GSH to acetylphenylhydrazine and the other drugs used.

Heparinated venous blood samples were used within 3 h of the time they were drawn. After determination of the red cell glutathione, 1 ml aliquots of whole blood were added to the tubes containing acetylphenylhydrazine, primaquine, aspirin, ibuprofen and flurbiprofen. The tubes were incubated at 37°C for 3 h after proper mixing. At the end of 3 h, the GSH concentration in test samples and in control samples was determined and compared.

Results

The results of the study are presented in Tables 2 and 3. The concentration of GSH was lower in G-6-PD deficient red cells than in normal cells (Table 2). The difference between the mean concentration of GSH in G-6-PD deficient and normal cells is statistically significant ($P < 0.05$). This has also been reported by various other investigators (Beutler, Dern, Flanagan & Alving, 1955).

The stability of red cells GSH to acetylphenylhydrazine and other drugs: Normal red cells incubated with acetylphenylhydrazine, primaquine, aspirin, ibuprofen and flurbiprofen showed reduction in red cells GSH levels which were similar to that seen with normal cells incubated without the drugs. G-6-PD deficient red cells incubated with aspirin, ibuprofen and flurbiprofen showed reduction in red cells GSH levels which were also almost similar to that seen with G-6-PD deficient cells incubated without the drugs. However, marked drop in red cells GSH levels was seen with acetylphenylhydrazine and primaquine compared to G-6-PD deficient red cells; incubated without the drugs (Table 2).

On comparing the effect of drugs on GSH of normal and G-6-PD deficient red cells it is seen that acetylphenylhydrazine and primaquine produced significantly more reduction in GSH levels in G-6-PD deficient red cells ($P < 0.02$). However, with the other drugs, this difference did not reach a level of statistical significance ($P > 0.05$), as the difference was very marginal (Table 3).

The red cells of G-6-PD deficient patients who gave a history of haemolytic reactions to sulfonamides showed marked fall in GSH levels after incubation with acetylphenylhydrazine and primaquine. The other drugs did not show any such effect.

Discussion

The development of glutathione stability test by Beutler (1957) has provided a method for the detection of individuals whose erythrocytes are susceptible to haemolysis by certain drugs, i.e. primaquine. A positive glutathione stability test is characterised by a marked decrease in the reduced glutathione content of sensitive erythrocytes after incubation of blood with acetylphenylhydrazine, whereas in normal erythrocytes, the glutathione content is but slightly decreased or unchanged. Subsequently, other investigators suggested that glutathione stability may be used as one of the models to screen drugs for possible haemolytic potential in G-6-PD deficient persons (Baxi *et al.*, 1978).

Table 2 Average GSH levels in normal and G-6-PD deficient red cells after incubation with drugs

Volunteers	Average GSH (mg/100 ml of red cells)									
	Blood alone		Acetylphenyl- hydrazine	Prima- quine	Aspirin		Ibuprofen		Flurbiprofen	
	Before	After			1	2	1	2	1	2
Normal	80.15 ±6.65	78.0 ±6.52	64.09 ±5.63	68.06 ±6.61	66.98 ±3.68	66.65 ±3.82	70.34 ±4.71	70.53 ±5.17	72.65 ±4.66	70.91 ±5.28
G-6-PD deficient	56.18 ±8.49	53.72 ±3.23	26.78 ±2.56	15.98 ±2.06	53.08 ±5.35	50.82 ±4.54	49.75 ±3.056	50.58 ±3.07	52.55 ±2.83	50.45 ±2.98

1 – low concentration, 2 – high concentration

Table 3 Comparison of effect of drugs on GSH of normal and G-6-PD deficient red cells (mean $\pm$ s.e. mean)

Drug	Percentage change		Mean difference	P*
	Normal	G-6-PD deficient		
Acetylphenylhydrazine	-17.5 $\pm$ 3.64	-48.6 $\pm$ 5.74	31.3	<0.02
Primaquine	-13.1 $\pm$ 2.94	-69.2 $\pm$ 4.55	56.1	<0.01
Aspirin (1)	- 4.4 $\pm$ 3.39	+ 1.1 $\pm$ 2.97	5.5	>0.05
Aspirin (2)	- 4.9 $\pm$ 3.32	- 0.7 $\pm$ 3.47	4.2	>0.10
Ibuprofen (1)	- 8.7 $\pm$ 2.88	- 5.3 $\pm$ 2.14	3.4	>0.10
Ibuprofen (2)	- 8.9 $\pm$ 1.95	- 5.5 $\pm$ 3.044	3.4	>0.10
Flurbiprofen (1)	- 5.1 $\pm$ 4.762	- 1.4 $\pm$ 2.94	3.7	>0.10
Flurbiprofen (2)	- 8.0 $\pm$ 3.41	- 7.1 $\pm$ 3.06	0.9	>0.10

1 – low concentration, 2 – high concentration, * – Mann Whitney 'U' test

In our study, we have also observed a marked decrease in GSH levels in G-6-PD deficient erythrocytes when incubated with acetylphenylhydrazine and primaquine. However, no such change was observed with drugs like ibuprofen, flurbiprofen and aspirin.

Acetylsalicylic acid (aspirin) has been reported to produce haemolytic reaction in some G-6-PD deficient persons. Though aspirin itself can induce haemolysis of G-6-PD deficient red cells, 2,5-dihydroxybenzoic (gentisic) acid, a known aspirin metabolite with redox properties, has been shown to play a major role in aspirin induced haemolysis (Shahidi & Westring, 1970). This explains the lack of change

demonstrated in GSH level in G-6-PD deficient red cells with aspirin, in our study. Even for certain other drugs, it is reported that the metabolite of the active drug is really a haemolytic agent (Goldstein *et al.*, 1969).

Ibuprofen and flurbiprofen have not been reported to produce any haemolytic reaction in G-6-PD deficient persons. The results of this *in vitro* study also indicate that they are relatively innocuous in such individuals.

This test seems to be useful for preliminary screening of haemolytic potential of drugs before undertaking any *in vivo* study.

References

- BAXI, A.J., BALAKRISHNAN, V., UNDEVIA, J.V., & SANGHVI, L.D. (1963). Glucose-6-phosphate dehydrogenase deficiency in Parsee community, Bombay. *Indian J. med. Sci.*, **16**, 493–500.
- BAXI, A.J., SONI, H.K. & VAIDYA, A.B. (1978). An *in vitro* model for screening haemolytic effects of drugs in G-6-PD deficient erythrocytes. In *Medical Genetics in India*, Vol. 2, ed, Verma I.C., pp. 215–221. Pondicherry: Auroma Enterprises Publication.
- BEUTLER, E. (1957). The glutathione instability of drug-sensitive red cells. *J. lab. clin. Med.*, **49**, 84–95.
- BEUTLER, E. (1969). Drug-induced haemolytic anaemia. *Pharmac. Rev.*, **21**, 73–103.
- BEUTLER, E., DERN, R.J., FLANAGAN, C.L. & ALVING, A.S. (1955). The hemolytic effect of primaquine. VII biochemical studies of drug-sensitive erythrocytes. *J. lab. clin. Med.*, **45**, 286–295.
- BEUTLER, E., DURON, O. & KELLY, B.M. (1965). Improved method for the determination of blood glutathione. *J. lab. clin. Med.*, **61**, 882–886.
- GOLDSTEIN, A., ARONOW, L. & KALMAN, S.M. (1969). Drug idiosyncrasy and pharmacogenetics. In *Principles of Drug Action*, 2nd edition, pp 442–452. New York: Harper & Row Publishers.
- SHAHIDI, N.T. & WESTRING, D.W. (1970). Acetylsalicylic acid-induced hemolysis and its mechanism. *J. clin. Invest.*, **49**, 1334–1340.
- WHO (1967). Standardization of procedures for the study of glucose-6-phosphate dehydrogenase. *Technical Report Series*, No. 366, 4–33.

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