Comparison of the Effect of Compound CDRI 80/53 [N-(3-acetyl-4,5-dihydro-2-furanyl)-N4-(6-methoxy-8-quinolinyl)1,4- pentanediamine] with Primaquine on Human Erythrocytes *In Vitro*

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ABSTRACT

Comparative *in vitro* study of a malaria antirelapse compound CDRI Code 80/53 with primaquine (PQ) was done on the erythrocytes of eight normal and six G-6-PD-deficient individuals. Reduced glutathione (GSH) was estimated in the RBCs exposed to the *in vitro* action of the drugs. Statistically significant decreases were observed at 25 µg/ml and 50 µ/ml doses of PQ as compared to the equivalent doses of compound 80/53 in both normal and G-6-PD-deficient erythrocytes. The study showed that compound CDRI 80/53 did not damage normal as well as G-6-PD-deficient erythrocytes to the same extent as PQ.

INTRODUCTION

It is documented that several well-tolerated drugs, viz. acetylphenylhydrazin, ascorbic acid, sulfonamides, nitrofurantoin, aspirin, chloramphanicol and phenacetin administered to some individuals having red cell abnormality, cause an acute haemolytic anemia due to an enzyme deficiency (3, 8).

Antimalarial drugs, such as primaquine (PQ) and other 8- aminoquinolines, are known to cause haemolytic episodes in subjects having glucose-6-phosphate dehydrogenase (G-6-PD) deficiency. The sensitivity of G-6-PD-deficient erythrocytes to PQ has been documented (1). In blood, reduced glutathione (GSH) is concentrated within the erythrocytes and is unstable when the red cells of sensitive individuals are exposed to the action of drugs, leading to a decrease in

the quantity of GSH (6). Estimation of GSH has been used as a measure to detect the *invitro* sensitivity of G-6-PD-deficient cells to drugs (5). In this study, a malaria antirelapse compound (13) [N-(3-acetyl- 4, 5-dihydro-2-furanyl)-N4-(6-methoxy-8-quinolinyl) 1,4- pentanediamine (CDRI code 80/53) was compared with PQ for its haemolytic action on normal and G-6-PD-deficient erythrocytes.

MATERIALS AND METHODS

The study was carried out using blood samples obtained from eight normal healthy volunteers and six G-6-PD-deficient individuals aged 15 to 50 years. G-6-PD deficiency was detected by the fluorescent spot screening test (10) and confirmed by the enzyme assay method (18).

Heparinised blood samples were collected from each individual and after centrifugation, the packed cells were washed three times with cold saline. One ml aliquots of washed cells were then incubated with different concentrations of the drugs ranging from 1 to 50 µg/ml base of PQ diphosphate and equivalent doses ranging from 1.25 to 62.5 µg/ml of compound 80/53 (calculated according to Puri *et al.* (6) in a water bath at 37°C with occasional agitation for 3 hr (15). GSH levels were estimated by the method of Beutler *et al.* (9).

RESULTS

Mean erythrocyte GSH levels in the controls (without drug) were significantly lower in the G-6-PD-deficient individuals (29.5±1.86 mg%) as compared to normals (49.91 ± 4.49 mg%).

Normal erythrocytes exposed to different doses of PQ showed a fall in GSH levels, which reached statistical significance at concentration 10 µg/ml, whereas the same incubated with compound 80/53 showed significant decrease in GSH levels only at concentration 31.25 µg/ml (Table 1).

In G-6-PD-deficient erythrocytes exposed to PQ, significant decrease in GSH levels was observed at concentration 5 µg/ml. Exposure to compound 80/53 decreased GSH levels significantly at an equivalent dose 6.25 µg/ml (Table 2).

At concentrations of 25 µg/ml and 50 µg/ml of PQ and equivalent doses of compound 80/53 in G-6-PD-deficient erythrocytes, the decrease in GSH level was statistically similar ($P < 0.001$) when compared to GSH levels in the controls.

Percentage decrease in GSH levels was more pronounced in normal and G-6-PD-deficient erythrocytes treated with PQ as compared to compound 80/53 (Figs 1 and 2). Statistically significant decreases were observed at concentrations of 25 µg/ml and 50 µg/ml of PQ as compared to the equivalent doses of compound 80/53 in both normal and G-6-PD-deficient arythrocytes (Tables 1 and 2).

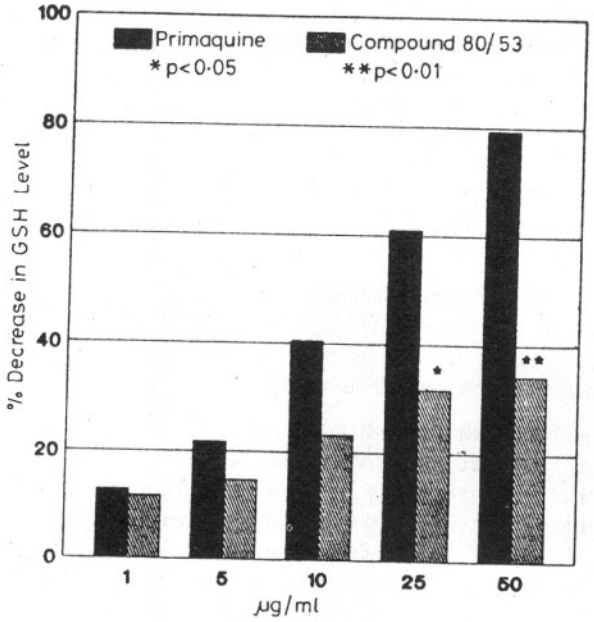

Fig. 1 – Percentage decrease in GSH levels of normal erythrocytes

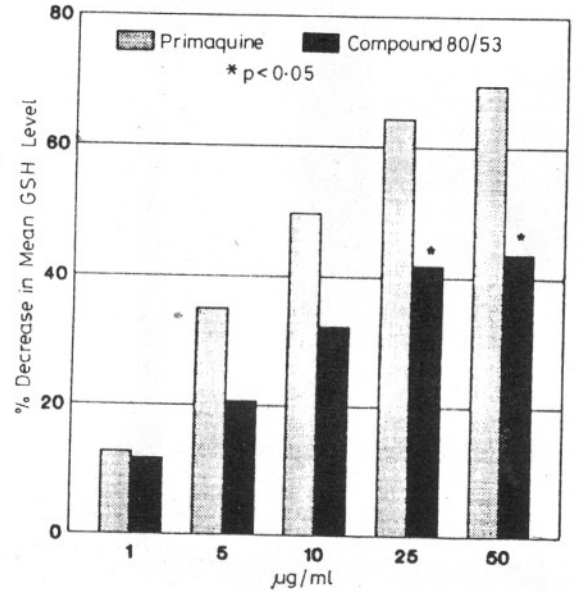

Fig. 2 – Percentage decrease in GSH levels of G-6-PD-deficient erythrocytes

DISCUSSION

Deficiency of G-6-PD is the commonest metabolic disorder of red blood cells and its prevalence is known to vary from place to place among different communities in India (12). It is a genetically determined red cell enzyme defect predisposing the subject to drug-induced haemolytic anemia (3, 4, 11). Several drugs used for common ailments, including antimalarials, are known to produce

Table 1 — GSH levels in normal erythrocytes with different doses of primaquine and equivalent doses of compound 80/53

Primaquine		Compound 80/53	
Dose (µg/ml)	GSH (mg%) Mean ±SE	Dose (µg/ml)	GSH (mg%) Mean ±SE
Control (No drug)	49.91±4.49	Control (No drug)	49.91±4.49
1.00	43.50±5.70	1.25	44.08±5.80
5.00	39.00±6.16	6.25	42.50±5.85
10.00	29.67±6.49	12.50	38.25±5.68
25.00	19.42±2.83	31.25	31.00±5.15*
50.00	10.37±1.57	62.50	32.75±5.39**

*Comparison of equivalent doses of compound 80/53 with primaquine
*P <0.05** P<0.01

Table 2 — GSH levels in G-6-PD-deficient erythrocytes with different doses of primaquine and equivalent doses of compound 80/53

Primaquine		Compound 80/53	
Dose (µg/ml	GSH (mg%) Mean ±SE	Dose (µg/ml)	GSH (mg%) Mean ±SE
Control (No drug)	29.50±1.86	Control (No drug)	29.50±1.86
1.0	25.75±2.17	1.25	26.04±2.20
5.0	19.17±1.50	6.25	23.42±1.66
10.00	14.83±1.89	12.50	20.00±1.73
25.00	10.50±1.52	31.25	17.17±1.81*
50.00	9.00±1.94	62.50	16.62±1.84*

* P<0.05. Comparison of compound 80/53 with primaquine

haemolytic anemia in individuals exhibiting G-6- PD deficiency. Toxic effects of PQ and 8-aminoquinoline compounds on haematological parameters have been reported by Beutler (1) and Sinton (4). Compound 80/53, a malaria antirelapse drug developed by CDRI, which is a derivative of PQ, was studied to compare its toxic effect with that of PQ.

Glutathione is considered important in the economy of erythrocytes. Several investigators have estimated GSH in erythrocytes to detect their sensitivity to the haemolytic action of drugs, such as PQ, aspirin and acetylphenylhydrazine (2, 7, 9, 15, 16). In this study, control GSH level in G-6-PD-deficient RBCs was found to be approximately 60% of the mean GSH level of normal RBCs, which is in agreement with the results of Beutler *et al.* (6). This shows that G-6-PD-deficient erythrocytes are deficient in GSH as well. Exposure to PQ decreased GSH levels in both normal and G-6-PD-deficient RBCs. Soni *et al.* (15) and Valame *et al.* (17), from their *in vitro* studies, have also shown a decrease in GSH levels in sensitive RBCs.

The decrease in erythrocyte GSH levels with compound 80/53 was not as marked as with PQ. This *in vitro* study shows that compound 80/53 does not damage normal and G-6-PD-deficient erythrocytes to the same extent as PQ.

ACKNOWLEDGEMENT

The authors wish to thank the Dean, Seth G.S. Medical College & KEM Hospital, for giving permission to carry out this study. Compound 80/53 was a gift from the Central Drug Research Institute (CDRI), Lucknow, CDRI also supported the work and the authors are grateful to the authorities of the Institute for this.

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