Brit. J. Pharmacol. (1958), **13**, 175

THE RÔLE OF QUININE IN HAEMOLYSIS

BY

R. S. GREWAL

From the Medical College, Nagpur, India

(RECEIVED FEBRUARY 1, 1958)

Quinine produces haemolysis of rabbit and human red blood cells in concentrations up to 1 in 700. In a concentration of 1 in 10,000 it increases the degree of haemolysis produced by various haemolytic agents like saponin, bile salts and digitonin *in vitro*. Quinine when injected intravenously in 35 mg./kg. dose in rabbits increases the susceptibility of red blood cells to the haemolytic action of saponin. Intravascular haemolysis following the administration of quinine is seen in cases of blackwater fever, and on the basis of experimental work described in the paper it is suggested that quinine precipitates these haemolytic episodes by rendering the red blood cells more susceptible to the action of tissue lytic factors.

The acid salts of quinine have a haemolytic action on erythrocytes in high concentration (Chopra, Mukerji, and Chopra, 1954), but these concentrations are not attained *in vivo* during the therapeutic use of quinine in cases of malaria. Kligler (1923) showed that dilutions of bile and quinine which separately had no effect on red blood cells caused prompt laking when used together. Mer, Birnbaum, and Kligler (1940) using ox bile as a haemolytic agent showed that the erythrocytes from malarial subjects were haemolysed more readily when quinine was given.

Intravascular haemolysis of erythrocytes during quinine administration is met with clinically in cases of blackwater fever. There is a considerable controversy about the aetiology of blackwater fever. It is now generally accepted that it is caused by infection due to *Plasmodium falciparum* and that administration of quinine and pamaquin precipitates an attack (Fairley and Murgatroyd, 1940). Innumerable instances from the literature can be cited where patients did not get haemoglobinuria until they had received a large dose of quinine; should the patient recover from the effects of the fever, a second dose of quinine may produce haemoglobinuria again. Fairley and Murgatroyd (1940) reported a case where four attacks occurred in one patient following the administration of quinine. Thus quinine under certain circumstances can cause haemolysis, but its mechanism of action has not been elucidated. The present work is an attempt to study the rôle of quinine in modifying the response of red blood cells to various haemolytic agents.

METHOD AND MATERIAL

The degree of haemolysis was estimated by a photoelectric colorimeter. The method is based on the principles of estimating total haemoglobin in a completely haemolysed blood sample and in the supernatant after the addition of different concentrations of haemolytic agents to the blood. The degree of haemolysis is then expressed in terms of total haemoglobin which represents 100% haemolysis; for example, if the total haemoglobin of whole blood is 15 g./100 ml., and if the supernatant after haemolysis gives a figure of 5 g., then the degree of haemolysis is 33.3%. The haemoglobin estimation was done by converting it into acid haematin by adding 0.1 N hydrochloric acid and the intensity of the colour developed was read off from the photoelectric colorimeter. The quantity of haemoglobin corresponding to a particular reading on the photoelectric colorimeter was determined by referring to a calibration curve prepared by plotting photoelectric colorimetric readings (% transmission) against the known concentrations of haemoglobin on a semilogarithmic graph paper. The solution containing a known concentration of haemoglobin was prepared from a blood sample, in which the haemoglobin content was determined by the blood iron method of Wong (1928). When readings were plotted as explained above, a linear relation was obtained between the haemoglobin concentration and the photoelectric colorimeter reading. From this curve the quantity of haemoglobin in a sample of blood could be read off directly when the colorimetric reading was known.

To set up an experiment the haemolytic agent was prepared in various dilutions in Simmel's fluid (sodium chloride 8.2 g., potassium chloride 0.2 g., magnesium chloride 0.2 g., calcium chloride 0.2 g., acid sodium phosphate 0.2 g., sodium bicarbonate 0.05 g., in 1 l.

distilled water). The total volume in each tube was 2.4 ml. In another set of tubes the same concentrations of the haemolytic agent, plus quinine dihydrochloride solution in a dilution of 1 in 10,000, were prepared, the total volume being again 2.4 ml. To all these tubes 0.1 ml. of heparinized blood was added. The tubes were kept at room temperature for 30 min. and were centrifuged at slow speed (1,500 to 2,000 rev./min.) for 15 min. The supernatant contained haemoglobin which was estimated in the form of acid haematin on the photoelectric colorimeter as described above.

Saponin (B.D.H.), digitonin (B.D.H.), bile salts (sodium tauroglycocholate, Bengal Immunity), quinine dihydrochloride (Howard) and heparin (Evans) were used.

Results

The range of haemolytic activity of quinine was determined for both rabbit and human erythrocytes. The percentage haemolysis caused by various concentrations of quinine in rabbit and human red blood cells is shown in Table I. Each

Table I

PERCENTAGE HAEMOLYSIS OF RED CELLS BY QUININE DIHYDROCHLORIDE

Conc. of Quinine	Rabbit	Human
1/100	78·0	73·4
1/200	55·5	68 0
1/300	31·2	59·0
1/400	18·0	48·6
1/500	7·5	31·4
1/600	4·0	12·2
1/700	Nil	3·3
1/800	Nil	Nil

estimate is the average of 10 experiments. From the table it is evident that quinine haemolysed rabbit red blood cells in concentrations up to 1 in 600 and human red blood cells in concentrations up to 1 in 700.

Experiments were next done to find out whether quinine in a concentration of 1 in 10,000 increased the degree of haemolysis produced by saponin. Table II gives the average of 15 experiments using

Table II

PERCENTAGE HAEMOLYSIS OF RABBIT BLOOD PRODUCED BY SAPONININ IN THE PRESENCE OF QUININE DIHYDROCHLORIDE

Conc. of Saponin	% Haemolysis	% Haemolysis in Presence of Quinine 1 in 10,000
1/15,000	77	90
1/20,000	72	85
1/30,000	38	63
1/40,000	16	32

the blood from 12 rabbits. From Table II, it is evident that quinine in 1/16 its haemolytic concentration increased the degree of haemolysis produced by saponin. Thus quinine rendered the cells more susceptible to the haemolytic action of saponin.

The work was extended to find out if quinine in a concentration of 1 in 10,000 modified the response of erythrocytes to other haemolytic agents. Table III gives the average % haemolysis

Table III

PERCENTAGE HAEMOLYSIS OF RABBIT RED CELLS BY BILE SALTS IN THE PRESENCE OF QUININE DIHYDROCHLORIDE

Bile Salt Conc.	% Haemolysis	% Haemolysis with Quinine 1 in 10,000
1/400	95·4	100
1/500	82·6	96·8
1/600	43·6	87·4
1/700	15·6	75·3
1/800	5·9	46·2
1/900	0·5	28

produced by bile salts (sodium tauroglycocholate) in ten rabbits. Table IV gives the average % haemolysis produced by digitonin in six rabbits.

Table IV

PERCENTAGE HAEMOLYSIS OF RABBIT RED CELLS BY DIGITONIN IN THE PRESENCE OF QUININE DIHYDROCHLORIDE

Digitonin Conc.	% Haemolysis	% Haemolysis with Quinine 1 in 10,000
1/100,000	92	100
1/120,000	86	94·2
1/140,000	56·8	79·2
1/160,000	42·5	65·1

The effect of quinine on the degree of haemolysis produced by bile salts on human erythrocytes was also studied. Table V gives the average

Table V

PERCENTAGE HAEMOLYSIS OF HUMAN RED CELLS BY BILE SALTS IN THE PRESENCE OF QUININE DIHYDROCHLORIDE

Bile Salt Conc.	% Haemolysis	% Haemolysis with Quinine 1 in 10,000
1/500	94·1	100·0
1/600	73·7	95·2
1/700	39·6	73·9
1/800	14·5	46·3
1/900	5·9	19·9

results obtained in ten experiments on different human blood samples. It is evident from the table that quinine increases the susceptibility of human erythrocytes to haemolysis *in vitro* in the same way as that of rabbit erythrocytes.

Experiments were done to see if quinine had a similar effect on red cell fragility when it was injected intravenously in a rabbit. The technique

was first to determine the degree of haemolysis by different concentrations of saponin added to blood of the animals *in vitro*. Then quinine dihydrochloride, 35 mg./kg. in 20 ml. of 5% glucose-saline solution, was injected intravenously at a slow rate. The percentage haemolysis produced by the same concentrations of saponin was determined on blood withdrawn ½ hr. and 1½ hr. after the quinine injection.

Table VI gives the average results of observations on 15 rabbits. It is evident that the intravenous injection of quinine increased the average

TABLE VI

PERCENTAGE HAEMOLYSIS OF RABBIT BLOOD BY SAPONIN AFTER INTRAVENOUS INJECTION OF QUININE DIHYDROCHLORIDE, 35 MG./KG.

Saponin Conc.	% Haemolysis Before Quinine	% Haemolysis ½ hr. after Quinine Injection	% Haemolysis 1½ hr. after Quinine Injection
1/20,000	81·3	90·4	96·3
1/25,000	62·4	64·7	82·3
1/30,000	45·9	52·1	69·2
1/35,000	32·5	43·5	65·8
1/40,000	27·3	33·9	53·5

percentage haemolysis produced by saponin. There were differences in the degree of haemolysis produced by a given concentration of saponin in different rabbits, but quinine increased the degree of haemolysis produced by a particular concentration of saponin in all cases except in 3 rabbits, in which quinine produced no increase in haemolysis. It was observed that quinine increased the speed, as well as the degree, of haemolysis.

DISCUSSION

The close association between administration of quinine in cases of malaria and occurrence of haemoglobinuria has been frequently noted. Foy and Kondi (1937b) found a high positive correlation between quinine administration and blackwater fever in Macedonia. Maegraith (1946) found a high incidence of haemoglobinuria during oral quinine therapy for malignant tertian malaria in the West African Command in 1941 to 1943. Findlay is quoted (Skipper and Haine, 1945) as stating that incidence of blackwater fever diminished considerably after the introduction of mepacrine in place of quinine for treatment and suppression of malaria. A similar lowering of incidence of haemoglobinuria was seen in the great malaria epidemic of 1942 in Greece when quinine was substituted by mepacrine for treatment of malaria (Foy and Kondi, 1950). This is specially remarkable since the relation between malarial incidence and blackwater fever was constant in previous years.

The factor responsible for the occurrence of intravascular haemolysis in blackwater fever has been postulated as a circulating haemolysin by Foy, Kondi, and Moumjidis (1941). Maegraith, Findlay, and Martin (1943) believe that there is a reduction of the normal inhibitory factor in cases of blackwater fever and that haemolysis is due to a shift to the lytic side of the balance between tissue lytic factor and its inhibitors. Though the factors responsible for causing intravascular haemolysis are known, the rôle of quinine in these haemolytic episodes has not been elucidated. On the basis of the experimental evidence presented in this paper it may be suggested that quinine can increase the degree of haemolysis by rendering the red blood cells more susceptible to the action of haemolytic agents. Recently it has been shown (Kärki, Burn, and Burn, 1957) that quinine diminishes the flux of ions in red cells. After storage for 3 to 5 days at low temperature the concentration of potassium within the cell falls and that of sodium rises. When these cells are incubated at 37° in the presence of glucose the concentration of potassium once more rises and that of sodium falls. These changes were found to be diminished by quinidine. Kärki (1958) has found that other antimalarial compounds have a similar action, though pyrimethamine has not. It is quite possible that this effect of quinidine on the permeability of the red cell membrane is connected with its tendency to favour haemolysis.

The investigation was supported by a grant from the Indian Council of Medical Research, for which the author wishes to express his gratitude.

REFERENCES

Chopra, R. N., Mukerji, B., and Chopra, I. C. (1954). *A Treatise on Tropical Medicine.* Calcutta: U. N. Dhur & Sons.

Fairley, N. H., and Murgatroyd, F. (1940). *Trans. R. Soc. trop. Med. Hyg.*, **34**, 187.

Foy, H., and Kondi, A. (1937b). Ibid., **31**, 123.

—— —— (1950). *Ann. trop. Med. Parasit.*, **44**, 309.

—— —— and Moumjidis, A. (1941). *Trans. R. Soc. trop. Med. Hyg.*, **35**, 119.

Kärki, N. T. (1958). *Arch. int. Pharmacodyn.*, **114**, 243.

—— Burn, G. P., and Burn, J. H. (1957). *Lancet*, **1**, 565.

Kligler, I. J. (1923). *Amer. J. trop. Med.*, **3**, 203.

Maegraith, B. G. (1946). *Trop. Dis. Bull.*, **43**, 803.

—— Findlay, G. M., and Martin, N. H. (1943). *Lancet*, **1**, 573.

Mer, G., Birnbaum, D., and Kligler, I. J. (1940). *Trans. R. Soc. trop. Med. Hyg.*, **34**, 373.

Skipper, E. W., and Haine, G. L. (1945). *Brit. med. J.*, **1**, 325.

Wong (1928). *J. biol. Chem.*, **77**, 409.