An improved method for removal of red cell-bound immunoglobulin using chloroquine solution

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In some patients with autoimmune hemolytic anemia or hemolytic disease of the newborn, the red cells are so heavily coated with immunoglobulin that phenotyping cannot be carried out unless the antibody is removed without destroying the red cell antigens. Studies were performed initially to determine the optimum conditions for removal of immunoglobulin from red blood cells (RBCs) using chloroquine. Group O, R_1r RBCs were coated with serial dilutions of anti-D; aliquots were incubated in chloroquine diphosphate (CDP) solution (200 g/L, pH 5.0) at 18°C, 25°C, 30°C, and 37°C, and tested by the antiglobulin technique at intervals of 30 minutes for up to 2 hours, the results being expressed as titration scores. These studies showed that antibody removal was much more efficient at 30°C and 37°C than at 18°C or 25°C. A further series of experiments was then carried out to assess the effect of chloroquine on red cell antigenicity. A 5 percent suspension of RBCs heterozygous for C, D, and E antigens, and for Kell, Duffy, and Kidd antigens, was incubated at 30°C and at 37°C in chloroquine solution. Aliquots were removed at 30-minute intervals for up to 2 hours, tested with serial dilutions of the appropriate antisera, and titration scores obtained. The antigens were well preserved after two hours of chloroquine treatment at 30°C. However, when treatment was performed at 37°C, antigenicity had markedly deteriorated by 60 minutes, although the antigens were still reasonably well preserved (except for Jk^b) at 30 minutes. It is therefore recommended that treatment with chloroquine solution prior to typing RBCs heavily coated with antibody should be carried out for 90 minutes at 30°C or for not more than 30 minutes at 37°C. ***Immunohematology*** 1994;10:22.

In autoimmune hemolytic anemia or hemolytic disease of the newborn, red blood cells (RBCs) are coated with warm-reacting antibodies and are susceptible to immune-type hemolysis. In severe cases, treatment with blood transfusions may be necessary, but investigation and selection of blood suitable for transfusion is sometimes hindered by an inability to type RBCs exhibiting a positive direct antiglobulin test (DAT). Although washing RBCs and using saline-agglutinating antisera enable the ABO and Rh phenotypes to be determined in most instances, some patient's RBCs are so heavily coated that removal of antibody is required before serologic tests can be performed.

Chloroquine and hydroxychloroquine have been used in the treatment of various immune diseases,[1-3] and laboratory studies show that these quinoline derivatives act by inhibiting antigen-antibody reactions.[2-5] Chloroquine has also been employed in removing red cell-bound antibody in vitro by breaking down disulfide bonds holding antigen-antibody complexes together. The cells are left intact with minimal antigenic loss and are thus suitable for blood typing.[6] Other methods of removing red cell-bound antibody prior to serologic testing have been advocated, such as ZZAP,[7,8] EDTA-glycine,[9] and heat elution.[10] However, all of these methods have serious drawbacks in comparison with chloroquine. ZZAP was found to be an excellent eluant,[7,8] but it denatured Duffy, MNSs, and Kell system antigens, although ABH, Rh, and Kidd typings were unaffected.[7] The EDTA-glycine method seems to have been only briefly reported.[9] It appears to offer a very quick and reliable means of removing red cell-bound IgG, while maintaining most of the antigens denatured by ZZAP; unfortunately, it also destroys Kell system antigens.[9] Heating antibody-coated RBCs for 5–30 minutes at 45°C or for 3–10 minutes at 50°C will sometimes dissociate enough antibody for cells to be typed by strongly reactive antisera;[10] however, this technique has the disadvantage of causing antigen weakening.[10] It would seem therefore that of all the methods considered, the chloroquine method is the most suitable for typing RBCs heavily coated with immunoglobulin. Previous work suggested that chloroquine treatment should be performed at room temperature,[6,11] but we have found that this is relatively ineffective and time consuming. In the current study, data will be presented to show that more efficient antibody removal can be obtained at higher temperatures without compromising red cell antigenicity.

Materials and Methods

Removal of red cell-bound antibody

To determine the optimum incubation time and temperature for effective removal of antibody from red cells using chloroquine, group O, R_1r RBCs were sensitized for

30 minutes with serial dilutions (neat to 1:128) of a strong IgG1 subclass anti-D (20 iu/mL). The RBCs were then washed four times with phosphate-buffered saline with a pH of 7.0. A small volume of RBCs was removed and tested with IgG anti-human globulin to assess the degree of sensitization. The sensitized RBCs had a 3+ DAT with neat anti-D, and still gave a visual reaction (1+) at 1:16. The strength of agglutination for each dilution of anti-D was given a numerical value, which was summed to produce a "titration score."[11] The remaining RBCs were divided into four aliquots, concentrated, suspended in chloroquine diphosphate solution (200 g/L, pH 5.0) to give a 5 percent cell suspension, and incubated at 18°C, 25°C, 30°C, and 37°C, respectively. A small volume of RBCs from each aliquot was removed at 30-minute intervals for up to 2 hours. The RBCs were washed four times in phosphate-buffered saline and tested with IgG anti-human globulin. The titration score was then recorded.

Effect of chloroquine on red cell antigenicity

Following the studies on the removal of red cell-bound antibodies, the effect of chloroquine treatment on selected RBC antigens was assessed at 30°C and at 37°C. Aliquots of unsensitized group O red cells heterozygous for C, D, and E antigens, and for Kell, Duffy, and Kidd antigens were placed in chloroquine solution to give 5 percent cell suspensions, and incubated at 30°C and 37° C. Small volumes of the suspensions were removed at 30-minute intervals for up to 2 hours, washed four times in phosphate-buffered saline, and tested with serial dilutions of the appropriate antibody by the serologic techniques for which these antisera had been standardized; the titration scores were then recorded.

Results

The results of the experiments to determine optimum chloroquine treatment for removal of red cell-bound antibodies are given in Table 1, while those for the effect of chloroquine on red cell antigenicity at 30°C and at 37°C are shown in Tables 2 and 3, respectively. Data in Table 1 show that antibody bound to RBCs can be removed by chloroquine and that the efficiency of this process depends on the temperature of treatment. Large quantities of immunoglobulin were removed after 30 minutes at 37°C and from 60 minutes onward at 30°C. If treatment was carried out at 18°C or 25°C, considerable amounts of antibody remained bound to RBCs, even after 120 minutes. These findings provided the rationale for the second series of experiments, which examined the effect of chloroquine treatment on RBC antigenicity. Table 2 shows that there was good preservation of all antigens after 120 minutes when treatment was carried out at 30°C. However, with treatment at 37°C, antigenicity had markedly deteriorated by 60 minutes, although it was reasonably well preserved (except for Jk^b) after 30 minutes (Table 3).

Table 1. Results of effects of time and temperature of chloroquine treatment on removal of red cell-bound antibody using anti-D and group O, R_1r red cells

Temperature at which chloroquine treatment was carried out	Time that antibody-coated cells were exposed to chloroquine			
	30 minutes	60 minutes	90 minutes	120 minutes
18°C	41*	22	16	10
25°C	35	26	18	14
30°C	21	8	2	1
37°C	6	0	0	0

*Titration score of tests using IgG anti-human globulin

Note: Pretreatment score = 48

Table 2. Effect of chloroquine treatment at 37°C for 90 and 120 minutes on red cell antigenicity

Reagent	Red cell phenotype	Titration score			Serologic techniques (carried out at 37°C)
		0 minutes (pretreatment)	90 minutes	120 minutes	
anti-D	R_1r	46	45	43	Saline
anti-C	R_1r	38	32	24	"
anti-E	R_1R_2	46	41	35	"
anti-c	R_1R_2	35	35	29	"
anti-e	R_1R_2	35	35	29	"
anti-K1	K1,2	65	63	63	IgG anti-human globulin
anti-K2	K1,2	35	37	31	"
anti-Fy^a	Fy(a+b+)	47	43	37	"
anti-Fy^b	Fy(a+b+)	31	33	26	"
anti-Jk^a	Jk(a+b+)	45	44	34	"
anti-Jk^b	Jk(a+b+)	23	24	23	"

Table 3. Effect of chloroquine treatment at 37°C for 30 and 60 minutes on *red cell antigenicity*

Reagent	Red cell phenotype	Titration score			Serologic techniques (carried out at 37°C)
		0 minutes (pretreatment)	30 minutes	60 minutes	
anti-D	R_1r	46	45	17	Saline
anti-C	R_1r	38	22	13	"
anti-E	R_1R_2	46	31	8	"
anti-c	R_1R_2	35	27	3	"
anti-e	R_1R_2	35	26	5	"
anti-K1	K1,2	65	47	8	IgG anti-*human* globulin
anti-K2	K1,2	35	21	5	"
anti-Fy^a	Fy(a+b+)	47	31	6	"
anti-Fy^b	Fy(a+b+)	31	31	5	"
anti-Jk^a	Jk(a+b+)	45	20	3	"
anti-Jk^b	Jk(a+b+)	23	6	0	"

Discussion

For typing RBCs heavily coated with immunoglobulin, chloroquine treatment should be carried out either for 90 minutes at 30°C or for not more than 30 minutes at 37°C (less time if Kidd antigen typing is required). It is important that RBCs of the appropriate type are treated and tested in parallel as positive and negative controls.

These recommendations differ from previous reports that advised treatment at room temperature.[6,11] Although these authors stressed efficiency of chloroquine treatment in removing red cell-bound immunoglobulin, it was noted that the antibodies were not completely dissociated in approximately 45 percent of cases, even after treatment for 120 minutes,[6] a finding consistent with the present results at 18°C (Table 1). Another report described a patient whose Rh antigens were found to be severely weakened after chloroquine treatment when tested with saline-reactive or chemically modified antisera.[12] The use of saline-agglutinating reagents did not appear to be a serious problem when chloroquine treatment was used within the time limits recommended in the present study (Tables 2 and 3), although the chloroquine technique was initially developed for blood typing by the indirect antiglobulin procedure.[13]

In summary, we conclude that typing RBCs heavily coated with antibody is best carried out after removal of bound immunoglobulins with chloroquine diphosphate solution (200 g/L, pH 5.0). Treatment for 90 minutes at 30°C or for not more than 30 minutes at 37°C is recommended so that antibody is efficiently removed without compromising red cell antigenicity.

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References

1. Forrester RM, Stratton F. A case of idiopathic autoimmune haemolytic anaemia in an infant treated with chloroquine. Vox Sang 1970;18:267-9.
2. Holtz G, Mantel W, Buck W. The inhibition of antigen-antibody reactions by chloroquine and its mechanism of action. Z Immunoforsch Exp Ther 1973;146:145-57.
3. Mantel W, Holtz G. Characterisation of autoantibodies to erythrocytes in autoimmune haemolytic anaemia by chloroquine. Vox Sang 1976;30:453-63.
4. Szilágyi T, Kávai M. The effect of chloroquine on the antigen-antibody reaction. Acta Physiol Acad Sci Hung 1970;38:411-7.
5. Mantel W, Holtz G. The inhibition of antigen-antibody reaction by quinoline derivatives. Z Immunoforsch Exp Ther 1974; 147:420-30.
6. Edwards JM, Moulds JJ, Judd WJ. Chloroquine dissociation of antigen-antibody complexes: a new technique for typing red blood cells with a positive direct antiglobulin test. Transfusion 1982;22:59-61.
7. Branch DR, Petz LD. A new reagent (ZZAP) having multiple applications in immunohematology. Am J Clin Pathol 1982;78:161-7.
8. James P, Rowe GP, Tozzo GG. Elucidation of alloantibodies in autoimmune haemolytic anaemia. Vox Sang 1988;54:167-71.
9. Kosanke J, McDowell MA, Stocker I, Thompson D, Wissel M. Treatment of DAT positive red cells with EDTA-glycine acid for antigen typing (abstract). Transfusion 1989;29(Suppl):57S.
10. Petz LD, Garratty G. Acquired immune hemolytic anemias. New York: Churchill Livingstone, 1980.
11. Issitt PD. Applied blood group serology. 3rd ed. Miami: Montgomery Scientific Publications, 1985.
12. Sassetti R, Nichols D. Decreased antigenic reactivity caused by chloroquine (letter). Transfusion 1982;22:537-8.
13. Moulds JJ, Edwards JM, Judd WJ. Decreased antigenic reactivity caused by chloroquine (letter). Transfusion 1982;22:538.

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