EDTA/glycine-acid versus chloroquine diphosphate treatment for stripping Bg antigens from red blood cells

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EDTA/glycine-acid (EGA) has been reported to remove IgG-bound antibodies from red blood cells (RBCs) and to denature Kell system and Er^a antigens. EGA-treated RBCs were tested in parallel with chloroquine diphosphate (CDP)-treated RBCs to evaluate whether EGA would remove Bg antigens from RBCs as efficiently as CDP. Fifty-seven serum/plasma samples containing known Bg antibodies were tested with untreated Bg+ RBCs, EGA-treated Bg+ RBCs, and CDP-treated Bg+ RBCs by an indirect antiglobulin test (IAT), using a low-ionic-strength additive solution and murine monoclonal polyspecific antiglobulin reagent. Of 57 samples, 40 (22 anti-Bg^a, 17 anti-Bg^b, and 1 Bg-related) were nonreactive by IAT with EGA-treated RBCs and CDP-treated RBCs, 14 (7 anti-Bg^a, 4 anti-Bg^b, and 3 Bg-related) were nonreactive by IAT only with EGA-treated RBCs, none were nonreactive in IAT with only CDP-treated RBCs, and 3 (anti-Bg^a) were still reactive by IAT with EGA-treated RBCs and CDP-treated RBCs. Therefore, EGA strips Bg antigens from RBCs. Our results indicate EGA treatment is more efficient and requires less time (1–2 minutes) to perform than the CDP procedure (30–120 minutes) for removal of Bg antigens from RBCs. ***Immunohematology* 1999;15:66–68.**

Key Words: EDTA/glycine-acid (EGA) treatment, chloroquine diphosphate (CDP) treatment, Bg antigens, Bg antibodies, indirect antiglobulin test (IAT)

EDTA/glycine-acid (EGA) treatment has been reported to remove IgG-bound antibodies from red blood cells (RBCs).[1,2] All common RBC antigens were reported to be detected on EGA-treated RBCs, with the exception of Kell system antigens.[1,3] Liew and Uchikawa[4] reported the loss of the high-incidence antigen Er^a when using EGA treatment. It also has been reported that EGA strips HLA antigens from platelets.[5]

Chloroquine diphosphate (CDP) treatment also has been reported to remove IgG-bound antibodies from RBCs.[6] The RBC membrane remains intact with no apparent loss of antigen reactivity. If incubation is carried out at 37°C for 30 minutes rather than at room temperature (RT), slight hemolysis and/or tanning of the RBCs and a decrease in antigen strength of M, Jk^a, Jk^b, Do^a, and Do^b blood group antigens may occur.[7-9] It has been reported that the CDP treatment strips Bg antigens from RBCs.[8,10] This procedure is useful in antibody identification when a patient's serum reacts unexpectedly with Bg+ commercial reagents or donor RBCs.

Because both the EGA and CDP techniques remove IgG-bound antibodies from RBCs, and CDP also strips Bg antigens from RBCs, our laboratory conducted a study to evaluate whether EGA would also remove Bg antigens from RBCs and be as efficient as CDP.

Materials and Methods

In our study, 57 serum/plasma samples containing known anti-Bg^a, -Bg^b, or Bg-related antibodies were tested with untreated Bg+ RBCs, EGA-treated Bg+ RBCs, and CDP-treated Bg+ RBCs in parallel by the indirect antiglobulin test (IAT). Each aliquot of Bg+ RBCs was treated with EGA according to the procedure previously described by Louie et al.[1] One volume of 10% EDTA was added to four volumes of glycine-HCl buffer (pH 1.5). To this mixture, one volume of washed, packed RBCs was added and incubated at RT for 1 to 2 minutes. One volume of 1.0 M TRIS-NaCl was added to adjust the mixture to a neutral pH. This procedure was performed without interruption to prevent EDTA precipitation or RBC hemolysis. After centrifuging, the supernate was removed and the RBCs were washed × 3 with physiologic saline. The washed RBCs were resuspended to a 3 to 4% suspension in physiologic saline or modified Alsever's solution (Gamma Biologicals, Houston, TX) for testing.

Another aliquot of each Bg+ RBC sample was also treated with CDP according to the manufacturer's directions. Four volumes of chloroquine diphosphate (Gamma-Quin™, Gamma Biologicals) were added to one volume of washed, packed RBCs. The tube was mixed

and incubated at RT for up to 2 hours. The mixture was washed × 4 with physiologic saline and resuspended to a 3 to 4% suspension in physiologic saline or modified Alsever's solution for testing.

The untreated Bg+ RBCs, the EGA-treated Bg+ RBCs, and the CDP-treated Bg+ RBCs were tested in parallel by the IAT. A standard low-ionic-strength (LISS) solution additive tube technique was employed.[11] Two drops of serum/plasma, one drop of a 3 to 4% RBC suspension (untreated, EGA-treated, or CDP-treated) and two drops of the LISS additive (Gamma Biologicals) were mixed together and incubated at 37°C for 30 minutes. All tests were washed × 4 with physiologic saline and murine monoclonal polyspecific anti-human globulin reagent (Gamma Biologicals) was added. All tests were inspected for macroscopic and microscopic agglutination. Reactivity was graded and recorded according to the methods described in the *Technical Manual* of the American Association of Blood Banks.[11] Microscopic reactions were graded like macroscopic reactions, i.e., based on the number of agglutinates present. All negative tests were confirmed by the addition of IgG-coated RBCs.

Results

Of the 57 samples containing Bg antibodies, 40 (22 anti-Bg^a, 17 anti-Bg^b, and 1 Bg-related) were nonreactive by IAT with EGA-treated RBCs and CDP-treated RBCs, 14 (7 anti-Bg^a, 4 anti-Bg^b, and 3 Bg-related) were nonreactive by IAT only with EGA-treated RBCs, none were nonreactive by IAT only with CDP-treated RBCs, and 3 (anti-Bg^a) were still reactive by IAT with EGA-treated RBCs and CDP-treated RBCs (Table 1). The strength of reactivity of the Bg antibodies with the untreated Bg+ RBCs ranged from microscopic to 2+ macroscopic by IAT.

Table 1. Results of EGA and CDP treatments of Bg+ red blood cells (RBCs)

Results	anti-Bg^a	anti-Bg^b	Bg-related antibodies	Total
Nonreactive with EGA- and CDP-treated RBCs	22	17	1	40
Nonreactive with EGA-treated RBCs, reactive with CDP-treated RBCs	7	4	3	14
Reactive with EGA-treated RBCs, nonreactive with CDP-treated RBCs	0	0	0	0
Reactive with EGA- and CDP-treated RBCs	3	0	0	3

Table 2 shows the 14 Bg antibodies that were nonreactive with EGA-treated RBCs but still reactive with CDP-treated RBCs. Seven of the antibodies exhibited the same strength of reactivity by IAT with CDP-treated RBCs as with untreated RBCs. The reactivity of the other seven antibodies was decreased by IAT with CDP-treated RBCs as compared with the reactivity with untreated RBCs. CDP did not completely remove the Bg antigens from the RBCs, whereas EGA appeared to completely strip the Bg antigens from the RBC membranes. Three examples of anti-Bg^a were still reactive by IAT with EGA-treated RBCs and CDP-treated RBCs.

Table 2. Bg antibodies reactive with CDP-treated RBCs by indirect antiglobulin test

Antibody	Case	Untreated RBCs	CDP-treated RBCs	EGA-treated RBCs
anti-Bg^a	B2368	1+	1+	0‡
	B0780f	1+^{w}	1+^{w}	0
	Marsden	2+	2+	0
	B0829	1+^{s}	0/3	0
	B2465	0/2*	0/1	0
	B2347	0/3	0/1	0
	Court	r†/4	0/1	0
anti-Bg^b	B2498	+^{w}	+^{w}	0
	62X04460	1+	1+	0
	B2510	r/4	0/2	0
	B2507	1+^{s}	r/4	0
Bg-related	B2418	2+	2+	0
	B2475	+	+	0
	B2449	1+	+	0

* 0/1 to r/4 (macroscopic and microscopic readings)
† rough
‡ macroscopic and microscopic readings nonreactive

Discussion

The EGA and CDP treatments strip Bg antigens from RBCs. Both the EGA and the CDP treatments have advantages and limitations. The advantages of EGA treatment are that it can be performed in less than 2 minutes and appears to be more efficient in the removal of Bg antigens than CDP. All three solutions for this treatment are commercially available as a kit and can be stored at RT. A limitation of the EGA treatment is that Kell system and Er^a antigens are also destroyed.

The advantages of CDP treatment are that it requires less manipulation and does not destroy Kell system and Er^a antigens. CDP also is commercially available, but it must be stored at 2° to 8°C. The limitations of this procedure are that CDP treatment may take up to 2 hours to strip Bg antigens from RBCs, and our data suggest it is not as efficient as EGA for removal of these antigens.

The three examples of anti-Bg^a that were still reactive

by IAT with EGA-treated RBCs and CDP-treated RBCs were only reactive with commercial reagent RBCs and/or donor RBCs that typed Bg(a+). It is possible that the Bg(a+) RBCs also were positive for another HLA-related RBC antigen, which was not stripped from the RBCs by either EGA or CDP treatments. If additional RBC samples from these Bg(a+) donors become available, complete HLA typing will be performed.

Acknowledgments

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References

1. Louie JE, Jiang AF, Zaroulis CG. Preparation of intact antibody-free red blood cells in autoimmune hemolytic anemia (abstract). Transfusion 1986; 26:550.
2. Byrne PC. Use of a modified acid/EDTA elution technique. Immunohematology 1991;7:46–7.
3. Julleis J, Sapp C, Kakaiya R. Glycine-EDTA as a substitute for AET in the inactivation of Kell System antigens on red blood cells (abstract). Transfusion 1992;32:14S.
4. Liew YW, Uchikawa M. Loss of Er^a antigen in very low pH buffers (letter). Transfusion 1987;27:442–3.
5. Sinor LT, Stone DL, Young R, et al. Comparison of chloroquine and acid elution methods for removal of platelet HLA antigens (abstract). Transfusion 1992; 32:14S.
6. Edwards JM, Moulds JJ, Judd WJ. Chloroquine dissociation of antigen-antibody complexes. Transfusion 1982;22:59–61.
7. McShane K, Cornwall W. Chloroquine reduces antigen strength (letter). Transfusion 1985;25:83.
8. Chen J, Spruell P, Moulds M. Studies using chloroquine diphosphate to strip HLA A and B antigens from red blood cells (RBCs) (abstract). New Orleans: ISBT/AABB Joint Congress 1990:152.
9. Beaumont AE, Stamps R, Booker DJ, Sokol RJ. An improved method for removal of red cell-bound immunoglobulin using chloroquine solution. Immunohematology 1994;10:22–4.
10. Swanson JL, Sastamoinen R. Chloroquine stripping of HLA A,B from red cells (letter). Transfusion 1985;25:439–40.
11. Vengelen-Tyler V, ed. Technical manual. 12th ed. Bethesda, MD:American Association of Blood Banks, 1996.

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