Case Report

Tolbutamide-induced Hemolytic Anemia

Paolo Malacarne, M.D., Giuseppe Castaldi, M.D., Maurizio Bertusi, M.D., and Giorgio Zavagli, M.D., Ferrara, Italy

SUMMARY

A 67-year-old female diabetic is presented who developed a Coombs'-positive hemolytic anemia after a year of treatment with tolbutamide. An IgG antibody was identified in the patient's serum that caused the agglutination of both the patient's red blood cells and tolbutamide-coated erythrocytes in the absence of complement. Such a reaction did not occur with the patient's erythrocytes when not exposed to tolbutamide. Agglutination of the patient's serum also occurred with erythrocytes treated with other sulfonylureas (chlorpropamide, glibenclamide, carbutamide) but not with phenacetin. DIABETES *26*:156-58, February, 1977.

Thrombocytopenia[1,2] and agranulocytosis, as well as pancytopenia,[3] have been reported to be consequences of oral hypoglycemic drug therapy with such drugs as tolbutamide and its derivatives and with the biguanides. However, only rare instances of oral hypoglycemic drug-induced hemolytic anemia have been identified.

Coombs' test positivity, with and without clinically overt hemolytic syndromes, has been produced by a wide variety of drugs.[4-8] A number of mechanisms have been postulated to account for Coombs'-positive hemolytic events.[4-10]

Logue et al.[11] reported a case of acute intravascular hemolysis in a diabetic patient treated with chlorpropamide due to the presence of an IgG antibody that, in the presence of the causative drug and fresh serum, caused lysis of complement-sensitive red cells obtained from patients with paroxysmal nocturnal hemoglobinuria. This antibody was found to cross-react with other sulfonylureas such as tolbutamide, carbutamide, acetohexamide, and tolazamide.

Bird et al.[10] have reported another case of hemolytic anemia associated with the development of the tolbutamide-reacting antibody. In this case, antibodies from the IgG class were also a factor but the reaction did not require the presence of complement. The antibody cross-reacted not only with glibenclamide- and chlorpropamide-treated red cells but also with phenacetin-treated red cells.

CASE REPORT

Mrs. M. S., aged 67, had been in good health until August, 1973, when, following the onset of thirst, throat dryness, and asthenia associated with polyuria, she was found to have diabetes mellitus. She was given insulin (until August, 1974) and then tolbutamide (Rastinon) at total daily doses as high as 1 gm. In May, 1975, following the onset of mild jaundice and anemia, the patient was admitted to a hospital where a diagnosis of "hemolytic anemia of unknown origin" was made. At home, jaundice reappeared, associated with marked weariness, and the patient was admitted to our institute in September of the same year.

On admission, the patient complained of marked fatigue, was pale, and presented mild scleral jaundice. Peripheral lymph nodes were not enlarged and neither hepatomegaly nor splenomegaly was noted. Her pulse rate was regular (88 beats per minute), blood pressure was 165/95 mm. Hg, and no other significant clinical signs were observed. At the time of admission (September 9th, 1975) laboratory findings were: hemoglobin 7.2 gm./100 ml.; red-blood-cell count 1,970,000/cu. mm.; PVC 22 per cent; MCHC 34 per cent. White-blood-cell count 9,800/cu. mm. with 72 per cent neutrophils, 3 per cent monocytes, 3 per cent

From the Istituto di Clinica Medica, University of Ferrara, 44-100 Ferrara, Italy.

Accepted for publication September 7, 1976.

eosinophils, 1 per cent basophils, and 21 per cent lymphocytes. Red cells were normochromic, and very few poikilocytes were observed. Heinz bodies were not identified after supravital staining. Platelets 180,000/cu. mm.; reticulocyte count 25 per cent; serum bilirubin 4.2 mg./100 ml.; direct bilirubin 0.4 mg./100 ml.; serum haptoglobin negative; serum iron 115 μg./100 ml.; TIBC 210 μg./100 ml. Immunoglobulin measurements were IgA 210 mg./100 ml. IgG 1,450 mg./100 ml., and IgM 200 mg./100 ml. Blood urea nitrogen was 37 mg./100 mg., total serum proteins 6.8 gm./100 ml., and electrophoretic protein pattern was within normal limits. No abnormal hemoglobins were found (Hb A_2 1.5 per cent). The direct Coombs' test was markedly positive (1:16,000). A sternal marrow biopsy demonstrated erythroblastic hyperplasia associated with marked macrocytosis.

The suspicion of a drug-induced hemolytic anemia was seriously considered. As tolbutamide was the only drug the patient had been taking for several months, this agent was stopped and replaced with insulin (18 U. daily of Novo Lenta, Copenhagen). After a few weeks, an increase in both hemoglobin levels (up to 13.4 gm./100 ml.) and red-cell count (up to 4,340,000/cu. mm.) could be observed. The patient was discharged on November 22nd of the same year.

Serologic Findings

Suspicion was directed toward tolbutamide as the hemolytic-anemia-inducing agent. Normal and tolbutamide-exposed compatible red cells were tested (a) under basal conditions, (b) devoid of complement activity, and (c) with tolbutamide-exposed patient's serum. For this purpose, 1 ml. of compatible red cells was incubated at 37° for 30 minutes with 0.5 ml. of an intravenous solution of 5 per cent tolbutamide. The red cells were then washed four times and resuspended in a physiologic saline solution to obtain a final 10-per-cent suspension. The patient's serum, when added in equal quantities, agglutinated the tolbutamide-exposed erythrocyte suspension but did not agglutinate the compatible tolbutamide-unexposed red-cell suspension. Heating the patient's serum at 56° for 30 minutes to destroy complement activity did not affect the above results. Previous exposure of the patient's serum to tolbutamide for 30 minutes inhibited any agglutination. Control tests performed with normal serum resulted in no agglutination; namely, a control's serum could not agglutinate tolbutamide-treated erythrocytes.

Tolbutamide-exposed red cells underwent evident agglutination after 30 minutes' incubation, but the reaction became more evident after one hour. The agglutinating antibody was identified as IgG by indirect Coombs' test using anti-IgG sera.

Further tests were performed to examine the capacity of other sulfonylureas to replace tolbutamide in the immune reaction with the antibody present in the patient's serum. The hemolytic system was tested with chlorpropamide, glibenclamide, and carbutamide. The antibody was found to cross-react with erythrocytes treated with the above-mentioned hypoglycemic agents, but it did not agglutinate phenacetin-exposed red cells.

DISCUSSION

Together with the observations of Logue et al.[11] and Bird et al.,[10] our report clearly demonstrated that sulfonylureas are capable of producing a hemolytic-anemia syndrome. Although in all reports to date the antibody involved has been identified as an IgG, the hemolytic mechanisms appear to be different. In the case reported by Logue et al.,[11] a stibophen-like (i.e., immune-complex) pattern occurred. In fact, both the clinical course and serologic reactions of the patient were consistent with what has been called an "innocent-bystander" reaction. The hemolytic syndrome was acute in onset and associated with severe intravascular hemolysis, and neither the drug nor the antibody was identified as being bound to red cells after several washings. Furthermore, complement was found to be necessary to induce "in-vitro" hemolysis.

In contrast both in our case and in that reported by Bird et al.,[10] the hemolytic mechanism seemed to involve hapten, as occurs in the penicillin-induced type of reaction. The drug did combine with the red-cell membrane and remained firmly bound. On drug exposure, the IgG antibody interacted with drug-coated erythrocytes, and this reaction did not require complement. Furthermore, both clinical signs and evolution of the illness were consistent with the pathogenetic mechanism.

Our case, as well as those reported by Logue et al.[11] and Bird et al.,[10] demonstrated that in cases of hypersensitivity to one sulfonylurea, the evoked antibody may cross-react with other compounds belonging to the same drug class. Thus, in these occurrences, treatment with all sulfonylureas must be stopped immediately.

REFERENCES

[1]Sauer, H., Fischer, K., and Landbeck, G.: Allergische Trom-

bozytopenie bei Tolbutamid-Behandlung des Diabetes mellitus. Med. Welt *13*:1899-903, 1962.

[2]Haller, H., and Stauzenberg, St. E.: Orale Diabetestherapie. Leipzig, Thieme Verlag, 1966, p. 257.

[3]Traumann, K. J., Grom, E., and Schwarzkopf, H.: Panzytopenie bei Diabetes-mellitus-Therapie mit Tolbutamid? Dtsch. Med. Wochenschr. *100*:250-51, 1975.

[4]Levine, B., and Redmond, A.: Immunochemical mechanisms of penicillin induced Coombs positivity and hemolytic anemia in man. Int. Arch. Allergy Appl. Immunol. *21*:594-606, 1967.

[5]Croft, J. D., Jr., Swisher, S. N., Jr., Gilliland, B. C., Bakemeier, R. F., Leddy, J. P., and Weed, R. I.: Coombs' test positivity induced by drugs: mechanisms of immunologic reactions and red cell destruction. Ann. Intern. Med. *68*:176-87, 1968.

[6]Pirofsky, B.: Autoimmunization and the Autoimmune Hemolytic Anemias. Baltimore, Williams and Wilkins, 1969, p. 342.

[7]Stefanini, M., and Johnson, N. L.: Positive antihuman globulin test in patients receiving carbromal. Am. J. Med. *259*:49-55, 1970.

[8]Henry, R. E., Goldberg, L. S., Sturgeon, P. E., and Ansel, R. D.: Serologic abnormalities associated with L-DOPA therapy. Vox Sang. *20*:306-16, 1971.

[9]Robinson, M. G., and Foadi, M.: Hemolytic anemia with positive Coombs' test: Association with isoniazid therapy. J.A.M.A. *208*:656-62, 1969.

[10]Bird, G. W. G., Eeles, G. H., Lichtfield, J. A., Rahman, M., and Wingham, J.: Haemolytic anaemia associated with antibodies to tolbutamide and phenacetin. Br. Med. J. *1*:728-30, 1972.

[11]Logue, G. L., Boyd, A. E., and Rosse, W. F.: Chlorpropamide-induced immune hemolytic anemia. N. Engl. J. Med. *283*:900-04, 1970,

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