Toxic Effects of Drugs on Erythrocytes

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ABSTRACT

The erythrocyte abnormality most often associated with the toxic effects of commonly used drugs is premature destruction. The mechanisms of erythrocyte destruction include: denaturation of unstable hemoglobin, oxidation of sulfhydryl groups in hemoglobin and the erythrocyte membrane in the presence of glucose-6-phosphate dehydrogenase deficiency, direct effects on enzymes, cholesterol or phospholipids of the erythrocyte membrane, and various autoimmune reactions.

Therapy includes stopping the drug and transfusions when anemia is severe. Splenectomy and steroids are rarely needed. A careful medical history and use of drugs only for good indications may avoid many of these reactions.

Introduction

Review of the toxic effects of drugs on erythrocytes reveals a number of mechanisms that lead to premature destruction of the cell and sometimes to obvious hemolytic anemia. Some of these mechanisms are well worked out and some remain obscure.

No attempt will be made to be comprehensive in covering all drugs that can potentially be involved. Recent reviews exist for further exploration. Drug reactions affecting production of erythrocytes will not be covered since this topic includes the vast field of chemotherapy and represents a major topic unto itself.

This paper will touch on some of the mechanisms of erythrocyte destruction involving hemoglobin, cell metabolism, the membrane, and immune reactions.

Toxic Effects Involving Hemoglobin

At last count, there were almost 100 hemoglobin variants, 80 percent of which affected the Beta chain of hemoglobin that led to instability of the molecule and premature denaturation. The amino acid substitution that leads to an unstable hemoglobin molecule is often in the vicinity of the heme pocket, but replacement of nonpolar by polar residues in the interior of the molecule also results in gross distortion of the protein. Deletions or insertions of additional amino acids in critical helical regions can also produce instability.

Many unstable hemoglobins have an increased susceptibility to oxidation to methemoglobin. The release of activated oxygen in the form of superoxide radicals and peroxide may accompany the reac-
Hemolytic episodes may be precipitated by the ingestion of oxidative drugs, particularly sulfonamides (table I). These drugs should be avoided in patients with unstable hemoglobins. Treatment, other than stopping the drug when anemia or jaundice appears, is not usually required. Transfusion may be lifesaving when severe anemia develops and splenectomy may be useful in patients with splenomegaly and severe anemia. Splenectomy probably should be avoided in patients with high affinity unstable hemoglobins since thrombosis sometimes follows splenectomy if the hemoglobin increases rapidly.

**Toxic Effects Involving Metabolism**

One of the earliest known evidences of drug induced hemolysis came from observations on Panamanian plantation workers being given quinine derivatives for malaria. The discovery that the hemolysis was seen in men with glucose-6-phosphate dehydrogenase (G6PD) deficiency came in the 1950's as a result of anti-malarial trials with Primaquine. Using $^{51}$Cr tagged red cells, it was possible for Beutler and coworkers to show the hemolytic effect of the drug was due to an abnormality intrinsic to the red cell and that the hemolysis was self-limited even if the administration of drug was continued. The latter observation was found to be a function of the age of the red cells. In G6PD deficient men, the older erythrocytes had defective enzymes and were quickly damaged by oxidant drug, whereas the younger erythrocytes, fresh from the marrow, had sufficient G6PD activity to survive for a longer period.

The mechanism of hemolysis appears to be similar to that for unstable hemoglobins. The lack of G6PD leads to a lack of reduced glutathione in the face of oxidative drugs. Cross linkages of sulfhydryl groups occur in both hemoglobin and membrane. Heinz bodies form and contribute to the rigidity of the red cell membrane resulting in early removal from the circulation.

In some of the more severe forms of G6PD deficiency, the hemoglobin may separate into free $\alpha$ and $\beta$ chains and intravascular hemolysis may be encountered. Some drugs produce a hemolytic reaction with milder forms of G6PD deficiency type A, (table I) while others produce hemolysis with only the more severe type B, or not at all.

Therapy consists of avoiding the oxidant drugs likely to cause hemolysis. Splenectomy is of little or no benefit. Transfusion may be necessary in severe G6PD deficiency with life threatening hemolysis.

**Toxic Effects Involving the Membrane**

Drug induced shortening of erythrocyte life-span may occur by one of two direct mechanisms. Chemicals may perturb the structure of the plasma membrane causing increased permeability, or they may penetrate to the intracellular space and alter cellular constituents and metabolism.

In recent studies, erythrocyte acetylcholinesterase (AchE) activity was measured in the blood of 36 alcoholic...
subjects and compared with the (AchE) activity in the blood of 41 normal controls. The activity in the hemolysates of alcoholic patients was significantly lower than that in the control subjects (69 ± 14 IU per gHb versus 145 ± 48 IU per gHb, p < 0.001, nonpaired t). Furthermore, in vitro incubation of ethanol/water mixtures with normal hemolysates or with whole erythrocytes showed significant inhibition of AchE activity at concentrations of ethanol above 100 mg per dl. The effect was immediate in hemolysates but was seen after a 15 hour incubation in intact erythrocytes. The authors washed the erythrocytes before measurement and used this as evidence that the inhibitory effect of ethanol was not a direct effect unless ethanol was irreversibly bound to erythrocyte membrane. This seemed unlikely since in three hospitalized alcoholic patients the AchE activity remained reduced for at least three days in the absence of alcohol.

Whether or not reduced levels of AchE activity is a cause of the anemia frequently seen in alcoholic patients awaits further research. The effects of ethanol on AchE in erythrocytes is similar to that seen in organophosphorus poisoning, which is associated with autonomic symptoms and rapid death owing to respiratory tetany. Hemolytic anemia has not been noted as a complication of organophosphorus poisoning.

Other studies in chronic alcoholic patients ingesting more than 150 g per day have demonstrated decreased membrane fluidity with an increase in cholesterol/phospholipid ratio. These changes were demonstrable even in patients showing normal MCV or minimal alterations in liver function tests and suggested that changes in erythrocyte membrane fluidity may indicate an early sign of alcohol effect not necessarily related to liver damage.

Eight alcoholic patients (48 to 75 years of age) were demonstrated to have normal liver function tests and absence of cirrhosis, but various degrees of steatosis by needle biopsy. Although target and spur cells were seen in the peripheral blood, no patient had remarkable reticulocytosis. Two patients had mean corpuscular volume (MCV) of 90 and 105 fl. No data on blood counts or hemoglobin were given.

The hydrophobic, fluorescent probe, diphenylhexatriene (DPH) was used to measure the degree of fluorescence polarization. Fluorescence polarization is related to the rotation freedom of the probe and provides a quantitative index of local membrane microviscosity. Ethanol in vitro enhances the fluidity of a number of membrane types, but chronic administration of ethanol to rats leads to decreased fluidity of membranes apparently related to compensatory changes in lipid composition.

In the alcoholic patients, there was a significant increase in the ratios of cholesterol/phospholipid and cholesterol/protein, while the ratios of phospholipid/protein were decreased. The membrane fluidity was statistically significantly increased compared with controls (p < 0.001). Membrane fluidity was altered even in patients showing normal MCV and liver function tests.

Treatment with N-5-methyl tetrahydrofolate was followed in all patients by a decrease in cholesterol/phospholipid ratio and by improvement in membrane fluidity that was statistically significant in two to three weeks. It appears that changes in erythrocyte membranes occur early in alcoholics before major functional or structural changes in the liver (except for fatty degeneration) and may reflect the diffuse effect of ethanol on biological membrane organization. Membrane fluidity correlates inversely with cholesterol/phospholipid ratio. Although this study suggests an important therapeutic effect of folate, an effect of abstinence from alcohol cannot be excluded.
Toxic Effects Involving Autoantibodies

There are three well established antibody mechanisms by which drugs can cause increased removal of erythrocytes from the circulation.

First, the drug can act as a hapten by attaching to the erythrocyte membrane and forming a neoantigen. Penicillin, given intravenously in high doses, will sometimes produce this form of hemolytic anemia. Anti-penicillin antibodies stimulated by the drug interact with the penicillin bound to the erythrocyte and coat the cell with IgG. The IgG coated cells are removed from the circulation by the macrophages in the spleen. Most individuals receiving penicillin will produce an IgM antibody response which does not produce a detectable hemolytic anemia. The IgG antibody responsible for the autoimmune hemolytic anemia occurs less frequently. Any antibody eluted from the erythrocytes in this setting reacts only with penicillin coated erythrocytes and shows no blood group specificity.

Second, the drug can act as antigen producing an IgM antibody, the interaction of which produces an immune complex which adheres to the erythrocyte membrane and activates complement causing intravascular hemolysis. Quinidine is a prime example of such a drug. In common with the hapten mechanism, the hemolysis occurs only in the presence of the drug; however, in contrast to the hapten mechanism the drug has low avidity for the cell membrane. Only small amounts of drug are necessary for hemolysis to occur and complement activation is the pathway to destruction. The antiglobulin test reacts only with anti-complement reagents.

Third, autoantibody induction has been described, most commonly as a result of treatment of hypertensive patients with alphamethyldopa, but recently with procainamide as well. The incidence of positive direct antiglobulin reactions (IgG) in patients taking alphamethyldopa vary from eight to 36 percent. Higher doses seem to induce more frequent antibody. The time to antibody appearance varies from three to six months. On the other hand, less than one percent of patients actually develop hemolytic anemia, and the mechanism appears to be splenic sequestration of IgG coated cells. Antibodies are often directed against Rh antigen similar to spontaneous autoimmune hemolytic anemia and presence of the drug is not necessary for serum antibodies or eluted antibodies to react with homologous cells bearing the appropriate antigens. It appears alphamethyldopa may interact with human T lymphocytes, resulting in loss of suppressor cell function and subsequent enhancement of the formation of autoantibodies by B lymphocytes.

Two explanations have been proposed for the small proportion of Coombs positive patient's who develop hemolytic anemia after receiving alphamethyldopa. One group has reported that patients with hemolysis have IgM antibodies and complement on their erythrocytes, while those without hemolysis have only IgG antibodies on their erythrocytes. The large amounts of IgM and Clq initially found were no longer detectable at the time of recovery from hemolysis.

Another report indicates impaired reticuloendothelial function is found in most patients treated with alphamethyldopa and hence, antibody coated cells are not removed from the circulation. The one (out of five) patient that had hemolysis showed rapid reticuloendothelial clearing of Chromium-tagged antibody coated cells, while those without hemolysis failed to show clearing of tagged autologous erythrocytes.

Procainamide autoimmune hemolytic anemia has been studied in 100 patients.
and controls at the Cedars Sinai Medical Center in Los Angeles.\textsuperscript{14} Antiglobulin tests were positive in 21 percent of patients and 10 percent of controls (p < 0.05). Only three patients developed anemia. The antibodies in procainamide patients were IgG and indistinguishable from those of autoimmune hemolytic anemia arising spontaneously.

In contrast, positive direct antiglobulin tests in controls were due to complement components. Red cell autoantibodies were not correlated with antinuclear antibodies nor with the Lupus-like syndrome described after long-term procainamide administration. \textit{In vitro} evidence suggests that procainamide, like alphamethyldopa, can differentially inhibit the activity of the cytotoxic-suppressor subset of T lymphocytes.\textsuperscript{18}

Therapy consists of discontinuing the offending drug and this is often the only therapy needed. Transfusions may be required in life threatening anemia, but glucocorticoids and splenectomy rarely play a role. Prognosis is good and hemolysis is mild in the vast majority of cases. Hemolysis usually stops within a few days of discontinuing the drug and the Coombs test becomes negative in the hapten and immune complex types of reactions. Even in cases of alphamethyldopa, hemolysis promptly ceases after cessation of the drug, although a positive direct antiglobulin test may remain for weeks or months.

\textbf{References}

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