Sensitivity to Hemolytic Drugs

An Inborn Error of Metabolism of the Red Cell

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THOSE charged with the care of the sick have often been dismayed to find that a drug innocuous to many, may produce catastrophic results in a few. Until recently, attempts to explain unusual reactions to drug therapy have ended in failure because untoward drug reactions usually occur sporadically and are often so dangerous that re-administration of the drug to the person manifesting the idiosyncrasy under study is out of the question. In 1953, my colleagues and I were presented with the unique opportunity to study in detail one such drug-sensitivity reaction, the hemolytic effect of the 8-amino-quinoline antimalarial compound, primaquine. These studies not only helped clarify the mechanism of sensitivity to this drug, but also led to the demonstration that a hereditary biochemical lesion of erythrocytes was responsible for many other drug-induced hemolytic anemias and for favism.[1]

The clinical manifestations of drug-induced hemolysis are well known.[2] Between one and three days after the patient begins to take the drug, the hemoglobin level of the blood falls, the urine darkens and the scleras become icteric. In severe cases, pain in the abdomen and the back may also occur. Usually, the patient recovers, but occasionally he succumbs, either to the severe anemia or to the nephropathy which follows massive blood destruction. In the case of favism, onset of hemolysis may be much more sudden, occurring within a very short period of time following the ingestion of fava beans.[3]

PRIMAQUINE SENSITIVITY

When the antimalarial compound, primaquine, was given to volunteer subjects, acute hemolytic anemia occurred in a few recipients. Others showed no evidence of hemolysis.[4] The now classic studies of Dern and his associates[5] demonstrated quite clearly that sensitivity to hemolysis was due to an intrinsic abnormality of the erythrocyte. When red cells from a drug-sensitive subject were labeled with Cr^{51} and given as a transfusion to nonsensitive recipients, the red cell survival time became remarkably shortened when the drug was administered. Conversely, red cells from a nonsensitive subject survived normally in the circulation of a sensitive person, even when the drug was administered to him and hemolysis resulted, as evidenced by a fall in the hematocrit.

Early in the course of studies of hemolytic anemia induced by primaquine therapy, a peculiar phenomenon was observed: when administration of the drug to a sensitive subject was continued, evidence of hemolysis abated spontaneously, hemoglobin levels returned to normal and the patient remained perfectly well, even when the dose of primaquine, which originally had elicited hemolysis, was doubled. Inspection of some of our survival curves suggested that the sensitivity to hemolysis might be a function of red cell age. Therefore, a primaquine-sensitive subject was given Fe^{59} so that a portion of his red cell population

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of narrow age range would be labeled.[6] Administration of the drug to this patient produced no hemolysis of labeled cells when the cells were aged from eight to twenty four days; however, when the cells were allowed to age longer in the circulation, a second course of drug administration (while the cells were sixty-three to seventy-six days of age) produced brisk destruction of labeled erythrocytes.

Earlier studies had demonstrated that persons who were sensitive to primaquine were also sensitive to other 8-amino quinoline antimalarials.[4] The Cr^{51}-labeling technic afforded us an opportunity to study the spectrum of drug sensitivity with a minimum of risks to the patient. Transfusion of red cells from sensitive and nonsensitive subjects were given to nonsensitive recipients and the rate of disappearance of red cells from the circulation was studied when the various drugs were given. In this way, it was determined that primaquine-sensitive red cells were also uniquely sensitive to the hemolytic action of a variety of other drugs, including sulfanilamide, acetanilid, phenylhydrazine, phenacitin and many others.[7] More recently, it has been found that nitrofurantoin,[8] and even aspirin when given in large doses,[9] may cause selective destruction of sensitive erythrocytes. It has also been learned that the red cells of subjects with sensitivity to the hemolytic effect of the fava bean are invariably primaquine-sensitive. The reverse, however, is not true. Primaquine-sensitive subjects frequently do not manifest the symptoms of hemolytic anemia when they are exposed to fava beans.[10]

It was clear, then, that persons who were primaquine-sensitive had red cells which were different from those of nonsensitive subjects. They were uniquely sensitive *in vivo* to the hemolytic effect of a variety of drugs. It was also clear that only the members of the red cell population of such persons were destroyed when the drug was given.

BIOCHEMICAL CONSIDERATIONS

But how did these cells differ from normal cells? When examined by classic hematologic technics, they appeared to be perfectly normal. Their morphology was normal on a wet-mount or unstained smear. No coating antibodies could be demonstrated. These cells were not uniquely sensitive to lysis *in vitro* by primaquine.[11] However, it was possible to show that the pattern of Heinz body formation in sensitive red cells is different from that of nonsensitive cells, when incubated under carefully standardized conditions with acetylphenylhydrazine.[12] Sensitive cells formed many small Heinz bodies in each cell, but sensitive cells, in general, formed only a few large Heinz bodies.

A number of biochemical investigations of primaquine-sensitive and nonsensitive red cells proved fruitless, but it was possible to demonstrate that the level of reduced glutathione (GSH) was rather consistently decreased in sensitive cells. However, we were not satisfied that glutathione deficiency was the primary defect of drug-sensitive cells, for the difference between normal and sensitive cells was small. There was also some overlap between sensitive and nonsensitive subjects when sufficient number of volunteer subjects were investigated, especially if only single determinations, rather than the average of several days determinations, were considered.

It was discovered that when drug-sensitive red cells were incubated with a reducing agent such as acetylphenylhydrazine, there was rapid destruction of red cell GSH; under identical conditions no GSH destruction occurred in nonsensitive erythrocytes.[13] I have called this test the glutathione stability test. It is a convenient way of demonstrating *in vitro* the presence or absence of drug sensitivity. It has also been of some help in further elucidating the basic metabolic disorder of these erythrocytes.

When GSH is destroyed in sensitive red cells under the influence of acetylphenylhydrazine, much of it is converted to the oxidized form of glutathione. Some is probably also bound to hemoglobin.[14] If we consider for a moment the mechanism by which GSH is normally maintained[15] in the reduced form in erythrocytes, we see that it is necessary for

TPNH to be present as a hydrogen donor in the reaction

$$GSSG^{*} + TPNH + H^{+} \rightleftarrows 2\,GSH + TPN^{+}$$

The red blood cell has two main pathways for the metabolism of glucose; the anaerobic, glycolytic pathway, and the direct oxidative pathway, or hexose-monophosphate shunt. It is in the latter pathway that TPN is reduced to TPNH. Investigations by Carson and others[16] of the three enzymatic steps, inhibition of which would result in failure to reduce oxidized glutathione properly, namely, glucose-6-phosphate dehydrogenase, phosphogluconic dehydrogenase or glutathione reductase, demonstrated that drug-sensitive red cells are deficient in glucose-6-phosphate dehydrogenase (g-6-pd). It has seemed likely that this is the primary defect of primaquine-sensitive red cells.

It is possible, however, and indeed there is now evidence to suggest that, at least in Sefardic Jews, the enzyme itself may be normal but that an activator is missing. If hemolysate from g-6-pd-deficient Sefardic Jews is incubated with normal stroma, there is marked activation of the enzyme.[17] Studies are under way in our laboratory to determine whether the same effect can be produced with the red cells of sensitive Negro subjects. We should like to determine whether stroma from Negro subjects with this red cell defect can reactivate enzyme in hemolysate from Sefardic Jews, since, as will be pointed out, there seem to be basic differences in this disorder between Caucasians and Negroes. Thus, it is possible that there may be mutual correction of the defect when stroma and hemolysate from Caucasian and Negro subjects are mixed.

A POSSIBLE EXPLANATION

Let us speculate briefly on how a deficiency of the GSH-reducing system might lead to destruction of red cells when a drug is administered. When a drug is given to a nonsensitive subject, we postulate that a reversible change in the hemoglobin molecule within red cells occurs. In the presence of GSH, this damage is repaired. In the process, GSH is oxidized to GSSG, but the latter compound is quickly restored to the reduced form, GSH, which now stands ready to protect the hemoglobin from assault by drugs. In the drug-sensitive cell, when GSH is oxidized in repairing the damage to the hemoglobin molecule, it is not again reduced to GSH because of the deficiency of glucose-6-phosphate dehydrogenase. Further, there is evidence to suggest that GSH synthesis is defective in sensitive cells.[18] As repeated assaults are made by the drug on the hemoglobin molecule, the damage becomes irreversible. Heinz bodies form within the red cells, and the cell is selected for destruction by the reticuloendothelial system in an, as yet, unknown manner. I must emphasize that this is only a hypothesis and that the exact mechanism of action of drugs in producing hemolysis of sensitive cells is as yet unknown. However, I believe that this hypothesis is a plausible one and is based on many findings.[14,19]

GENETIC ASPECTS

It has been established clearly that primaquine sensitivity is genetically transmitted. The mode of transmission has been elucidated by the studies of Childs et al.[20] Szeinberg and his associates,[10] and by others.[21] The defect is a sex-linked one and is transmitted presumably on the X chromosome, from the mother to her daughters or to her sons, but by the father only to his daughters. The penetrance of the gene for primaquine sensitivity is variable in the female. In the affected male, the full-blown typical enzyme deficiency and drug sensitivity is present. However, in the female, having one chromosome carrying the defective gene and one carrying the normal gene, the expression may vary from full expression to no detectable expression at all. It is presumed that the homozygous female invariably has the severe defect.

The distribution of this defect is now known to be worldwide.[21] It is particularly common in areas in which the incidence of malaria is high. Motulsky[22] has suggested that this defect may have survived in these populations by conferring an increased resistance to malaria. The severity appears to differ in Caucasian, as compared with Negro populations who are

* Oxidized glutathione.

affected.[23] It may be that two separate mutations have arisen in these populations, both with the same final effect but conferring varying degrees of enzyme deficiency. In addition, a biochemically similar, but perhaps more severe, defect has been observed among certain subjects with nonspherocytic congenital hemolytic anemia.[24] Thus, a third mutation appears to result in so severe a defect in the enzymatic activity of erythrocytes that clinically apparent shortening of red cell survival occurs.

Since g-6-pd deficiency is genetically determined one might suspect that severe depletion of this enzyme would be found in all somatic cells. While it has been possible to demonstrate deficiency of this enzyme in various cell types of Caucasian subjects,[25,26] this has been more difficult to achieve in Negro subjects with this abnormality.[27] It is possible that the enzymatic deficiency is particularly severe in erythrocytes because they are nonnucleated and, therefore, do not have the capacity to synthesize protein once they are launched from the marrow to complete their 120 day life span in the circulation. Indeed, it has been demonstrated[28] that the activity of g-6-pd diminishes in normal erythrocytes as they age and virtually disappears from the erythrocytes of those with g-6-pd-deficiency. This probably explains the greater susceptibility of older red cells to hemolysis.

Much remains to be learned about the exact nature of this defect and the way in which it renders affected persons sensitive to the hemolytic effect of drugs. Much, however, has already been learned and the road of exploration has been exciting.

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