Glucose-6-Phosphate Dehydrogenase

In Vitro Correlated with In Vivo Activity and Reticulocytosis

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ABSTRACT

In vitro activity of erythrocyte glucose-6-phosphate dehydrogenase (G-6-PD) does not always correlate with in vivo hemolytic manifestations. Many of these non-correlations are reviewed and can now be explained on the basis of altered substrate affinity and/or altered inhibition by intracellular metabolites. These alterations are not detected by standard assay conditions. The influence of a young erythrocyte population upon in vitro G-6-PD activity was determined and the lower limit of expected values shown to be raised at least to the mean of an average age erythrocyte population.

Introduction

The erythrocyte glucose-6-phosphate dehydrogenase (G-6-PD) is a NADPH-producing enzyme of the hexosemonophosphate pathway, highly important in maintenance of membrane glutathione in the reduced state as a protector against peroxides and oxidant drugs. The x-linked locus for this enzyme is highly mutable, with over 100 known variants. One variant, the African, or A−, type occurs in 12 percent of the black male population, thus constituting a potential hemolytic problem for a large population.

As reviewed by Luzzatto, activity of erythrocyte G-6-PD normally declines with erythrocyte age until at 120 days of age, the cell contains about 2 percent of the NADPH generating capability which it had when it entered the circulation. This activity (B type enzyme) is sufficient so that red cell survival is not compromised.

A cell containing the African variant, A−, can enjoy at least 75 percent of its normal lifespan under basal conditions. By this time, the cell contains about 0.2 percent of its original NADPH generating capacity, and the reticuloendothelial (RE) system removes it. Usually, the shortening of the red cell lifespan is so minimal that it can be fully compensated by a slight increase in bone marrow output. The estimated 100
million persons carrying this A\textsuperscript{−} mutant G-6-PD should never demonstrate anemia in absence of triggering factors such as drugs or infection. Cells carrying a different variant of G-6-PD, such as Gd Oklahoma or Gd Albuquerque, reach the 0.2 percent low level of NADPH production capacity at which they are removed by the RE system long before they have completed half their lifespan; a chronic non-spherocytic anemia results.

In the presence of an oxidant stress, NADPH production is increased by shunting glucose from the glycolytic, energy-producing, pathway through the hexosemonophosphate pathway. This stress also produces an increased demand for NADPH, so that the "threshold value" of NADPH production, below which the cell is removed by the RE system, is set at a new value ten-fold higher.

In this instance of oxidant stress, the B (normal) enzyme is quite adequate, 60 percent of the A\textsuperscript{−} variant cells will still survive, and only 25 percent of the more severe variant cells will survive the oxidative insult. The degree of hemolysis, both in absence and presence of stress, depends primarily and critically on the rate of decline in enzyme activity with red cell age, which is characteristic for each variant of G-6-PD.

Of the approximately 100 variants discovered in the last 20 years, about 40 of them have normal activity or mild enzyme deficiency and are associated with no clinical manifestations. About 20 variants are associated with chronic non-spherocytic
hemolytic anemia. The rest have severe enzyme deficiency in erythrocytes but require an oxidative insult for hemolysis to occur.11

Yoshida11 and Kirkman4 demonstrated that the hemolytic manifestation of variants such as Gd Bat-Yam, Gd Ramat-Gan, and Gd Worcester, can be easily correlated with the extremely low in vitro activity of the variant G-6-PD. Other variants, such as Gd Oklahoma, Gd Ashdod, and Gd Freiburg, also were chronically hemolytic, despite much greater in vitro G-6-PD activity. Their hemolytic state appears to be due to their low affinity (high K_m) for the glucose-6-phosphate (G-6-P) substrate. Some variants, such as Gd Albuquerque, Gd Milwaukee, Gd Glichy and Gd Strasbourg appear to hemolyze chronically owing to both low in vitro activity and low G-6-P affinity. Gd Manchester, Gd Alhambra and Gd Tripler hemolysis could not be explained by either low activity or low substrate affinity. These mutant enzymes were shown to have an altered inhibition constant (K_i) for NADPH,—that is, they are especially sensitive to the relatively high NADPH intracellular concentration. Therefore, in the presence of the high concentration of NADPH, as in the erythrocyte, these hemolytic variants can scarcely function. The classical in vitro assay, as recommended by the World Health Organization10 offers conditions just the reverse to the in vivo—saturating NADP and no NADPH. Under these conditions, altered K_i mutants can still express G-6-PD activity in vitro of greater than 20 percent of normal. Gd Union, a non-hemolytic variant with quite low in vitro activity, conversely appears to have both a higher G-6-P substrate affinity (lower K_m) and is more resistant to NADPH inhibition. Thus, our in vitro non-physiologic assay conditions can provide very misleading patho-physiologic information.

In 1967, the World Health Organization published criteria and methods for classifying G-6-PD variants, which include electrophoretic migration in starch gel, K_m for G-6-P, activity with a substrate analogue and thermal stability.10 To this list must now be added the K_m for NADP and the K_i for NADPH and for ATP.

There has been recent literature controversy concerning whether altered K_i for NADPH can truly explain hemolysis resistance in instances when intracellular NADP/NADPH ratios do not appear to support this argument.5,6,12 This problem will most likely not be resolved until more variant cells have been assayed for these parameters.

Another point which must be considered when interpreting results of in vitro G-6-PD assay is reticulocytosis. Marks et al8 demonstrated in the late 1950’s that young red cells and reticulocytes can have as much as three times the activity of average age red cells. It is now well recognized that removal of older deficient cells by a hemolytic event and addition of younger more G-6-PD active cells can yield an erythrocyte population which will not demonstrate G-6-PD deficiency as the etiology of the hemolytic event. Classic hematology texts recommend that, in such instances, one should delay the assay until such time as reticulocytosis subsides. This is not always feasible, as in the case of a chronic non-spherocytic anemia where the reticulocytosis will never subside. Additionally, during this waiting period, if G-6-PD deficiency is only mildly suspect, the patient may well be exposed further to crisis precipitating drugs such as the sulphonamides, vitamin K analogues, antipyrine, aspirin or antibiotics. As infection itself is an oxidant stress, the likelihood of one of these agents being administered is quite great.7

The G-6-PD activity was measured of washed erythrocytes from patients on whom a reticulocyte count had been requested, i.e., a potentially hemolytic population. These results are shown in figure 2.
Several points should be made. First, those patients with a reticulocyte count below 1.5 percent (generally considered normal) when taken from a suspect hemolytic population have a higher expected range than do blood donors and laboratory volunteers. These younger cells cannot be visually detected by a reticulocyte count. Secondly, the G-6-PD activity does not increase linearly with initial reticulocyte count. This probably reflects preferential removal of larger reticulocytes from the upper portion of the cell layer during washing. Three patients subsequently proven to be G-6-PD deficient have had at least one assay within the normal range, as shown by (—) in figure 2.

Although one cannot apply a "reticulocyte correction" to the measured G-6-PD activity, it can be stated that if a suspect G-6-PD deficient with reticulocytosis does not at least demonstrate G-6-PD activity at or above the normal mean, he should be a prime candidate for a true deficiency.

Summary

G-6-PD is the product of a highly mutable locus. Its deficiency will not always be reflected in the standard assay, owing to the presence of reticulocytes and/or to the extreme differences in in vivo environment and in vitro assay conditions.

With limited resources, the major guide as to when to extend the laboratory time and effort to detect and classify these many variants must rest heavily on the physician's degree of suspicion.

References