

Henna: A Potential Cause of Oxidative Hemolysis and Neonatal Hyperbilirubinemia

William H. Zinkham, MD and Frank A. Oski, MD

ABSTRACT. *Objective.* To evaluate the *in vitro* oxidative potential of lawsone (2-hydroxy-1,4 naphthoquinone). Lawsone is a chemical present in henna, the crushed leaves of which are used worldwide as a cosmetic agent to stain the hair, skin, and nails.

Methodology. Venous blood from glucose-6-phosphate dehydrogenase (G6PD)-normal and G6PD A⁻ subjects were incubated with various amounts of lawsone for 2 hours at 37°C. Reduced glutathione and methemoglobin (MHb) levels were measured before and after incubation.

Results. Final molar concentrations of lawsone in normal blood of 1.4, 2.8, 5.7, and 8.6 × 10⁻³ mol/L increased MHb percentages from 0.5% to 2.2%, 8.3%, 9.5%, and 12.5%, respectively. In a G6PD A⁻ blood, MHb percentages were 19.8%, 32.2%, 44.9%, and 53.9%. At a lawsone concentration of 2.8 × 10⁻³ mol/L, blood from 15 healthy adults formed MHb percentages of 7.4% ± 3.3% (±1 SD); in blood from 4 G6PD A⁻ adults, percentages were 44.5%, 40.6%, 41.3%, and 42.8%. Simultaneous measurements of reduced glutathione revealed preincubation values of greater than 40 mg/100 mL of red cells in blood of healthy and G6PD A⁻ subjects. Postincubation values were greater than 40 in blood of healthy subjects and less than 40 in blood of G6PD A⁻ subjects.

Conclusions. These *in vitro* observations indicate that lawsone is an agent capable of causing oxidative hemolysis. In regions of the world where there is a high incidence of G6PD deficiency and unexplained hyperbilirubinemia, oxidative hemolysis secondary to the cutaneous application of henna could be the initiating event. *Pediatrics* 1996;97:707-709; *neonatal hyperbilirubinemia, henna, lawsone, glucose-6-phosphate dehydrogenase, oxidative hemolysis.*

ABBREVIATIONS. G6PD, glucose-6-phosphate dehydrogenase; GSH, glutathione; MHb, methemoglobin.

Numerous reports from Greece, Israel, India, Thailand, Ghana, Nigeria, Turkey, Sardinia, and China indicate that neonatal hyperbilirubinemia, especially in glucose-6-phosphate dehydrogenase (G6PD)-deficient infants, is associated with significant morbidity and even mortality.^{1,2} A variety of causes have been suggested: exposure to environmental factors such as naphthalene, menthol, breast milk of mothers who have ingested fava beans, immaturity of the liver,

and the nature of the mutational event affecting G6PD activity. However, the pathophysiology of the disorder remains unknown in many of the infants.

During a medical tour of the Middle East, one of us noted that some of the infants with unexplained neonatal hyperbilirubinemia had staining of the skin after the topical application of henna (F. A. Oski, unpublished observation, 1993). Henna is a dye, the source of which is the shrub *Lawsonia alba*. The plant is grown extensively in the Middle East and Africa, and large amounts are imported into the United States. When the dried leaves are soaked in water and applied to the hair, skin, or nails, an auburn to red color develops, hence, its worldwide use as a cosmetic agent. In some countries, ceremonial and social events, including weddings and circumcisions, are celebrated by the application of henna to the skin to create a variety of designs and patterns. Approximately 100 g of henna are needed to stain the hands and feet of an adult, and the dyeing process requires 6 hours or more.³

An important chemical ingredient of henna is lawsone (2-hydroxy-1,4 naphthoquinone), constituting about 1% by weight of the crushed leaves.⁴ The structure and redox potential of lawsone is similar to that of one of the naphthalene metabolites, 1,4-naphthoquinone, a potent oxidant of G6PD-deficient red cells.⁵ Because of these similarities, together with the knowledge that percutaneous absorption of naphthalene may hemolyze G6PD-efficient red cells, studies were designed to determine whether lawsone also may cause oxidant injury to red cells.

METHODS

Venous blood from healthy adult volunteers was collected in tubes containing ethylenediaminetetraacetic acid K₃ (Becton-Dickinson Co, Rutherford, NJ). Lawsone, 250 mg, was added to 25 mL of methanol. After pipetting various amounts of lawsone to a series of test tubes, the methanol was evaporated by exposure to room air for 2 days or by heating the tubes to 85°C for 5 minutes. All tubes and solutions containing lawsone were stored in the dark. After incubating whole blood with lawsone for 2 hours at 37°C, the red cells were washed three times with isotonic saline (15 volumes of saline to 1 volume of whole blood) and resuspended in isotonic saline to a final hematocrit of approximately 35%. Reduced glutathione (GSH) and methemoglobin (MHb) levels were measured before and after incubation. Measurements of hemoglobin, MHb, GSH, and G6PD were determined by methods previously described.^{6,7}

RESULTS

Whole blood from 15 adults with normal G6PD activity and 4 G6PD A⁻ subjects was incubated with lawsone in a final concentration of 2.8 × 10⁻³ mol/L.

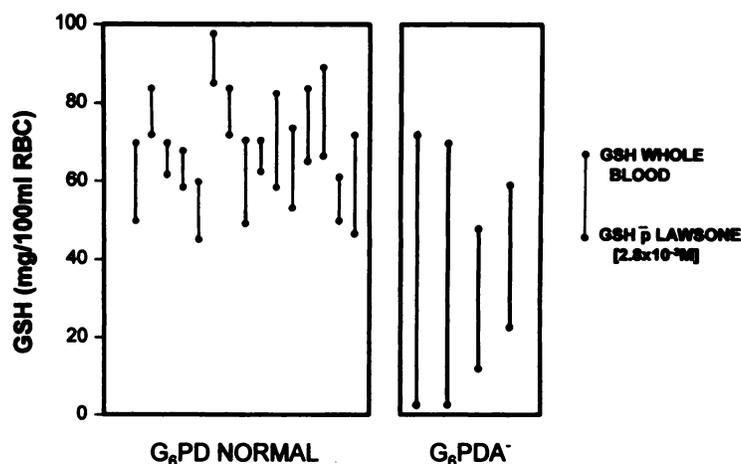
From the Department of Pediatrics, Johns Hopkins University School of Medicine, and Johns Hopkins Hospital, Baltimore.

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Reprint requests to (W.H.Z.) Department of Pediatrics, Brady 210, Johns Hopkins Hospital, Baltimore, MD 21205.

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Fig 1. Preincubation and postincubation GSH values in blood of 15 G6PD-normal and 4 G6PD A⁻ subjects exposed to a final concentration of lawsone of 2.8×10^{-3} mol/L.



In healthy adults MHB percentages were $7.4\% \pm 3.3\%$ (± 1 SD), and in the G6PD A⁻ subjects, the percentages were 44.5%, 40.6%, 41.3%, and 42.8%. The range of preincubation MHB percentages for all subjects was 0.4% to 0.6%. Preincubation and postincubation values for GSH are plotted in Fig 1.

Figure 2 illustrates the degree of MHB formation in G6PD-normal and G6PD-deficient blood exposed to various concentrations of lawsone. The rate of MHB formation is approximately threefold to fourfold greater in G6PD A⁻ blood than in normal blood.

DISCUSSION

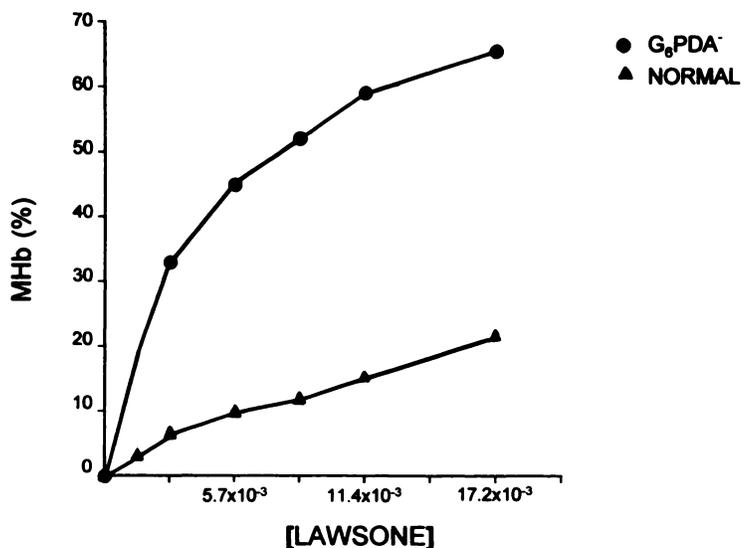
These in vitro observations indicate that lawsone, a chemical constituent of henna, is capable of inducing oxidative injury to G6PD-normal red cells, and even more so to G6PD A⁻ red cells. Furthermore, concentrations of lawsone producing oxidative changes are similar to those reported for the naphthalene metabolites α - and β -naphthol and α - and β -naphthoquinone, documented causes of severe hemolysis in G6PD-deficient subjects.⁸ The amount of henna used to stain the skin of newborns is unknown. If 100 g is required to stain the palms and soles of adults, and assuming that the surface area of the newborn is approximately $\frac{1}{10}$ that of an adult, then it is reason-

able to suggest that the infant would be exposed to 10 g of henna containing 1 g of lawsone. Thus, the percutaneous absorption of henna could be a possible source of oxidative injury to neonatal red cells.³

Other factors contributing to the oxidative effects of henna are the biochemical characteristics of the infant's red cells. For many years, a variety of studies have demonstrated the unique vulnerability of the newborn's red cells to oxidant-induced injury.⁹ Similar observations have originated from studies of G6PD-deficient red cells, an inherited defect, which has revealed the hemolytic potential of many drugs and chemicals, including naphthalene. In those parts of the world where traditional and ceremonial use of henna exists, there is a very high incidence of G6PD deficiency.¹⁰ Thus, the chances of henna-induced hemolysis are greatly enhanced, especially in G6PD-deficient infants. Therefore, the role of henna as one of the causes of unexplained neonatal hyperbilirubinemia merits further exploration by the performance of appropriate clinical and laboratory studies.

For many years, henna has been used for the treatment of skin disorders, many of which may be fungal in origin. Because of the presumed antifungal properties of henna, together with its structural similarity to 5-hydroxynaphthoquinone, a potent fungicide,

Fig 2. Effects of increasing concentrations of lawsone on MHB percentage in G6PD-normal and G6PD A⁻ blood.



henna powder was administered orally to four patients with intestinal moniliasis. No untoward effects were noted at a dose of 2 g three times a day for 1 month.¹¹ Obviously, additional studies are necessary to determine whether henna is nontoxic after oral administration. As is true for naphthalene and its metabolites, hemolysis may not occur unless the subject's red cells are deficient in G6PD.

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CONFESSIONS OF THE "OBSERVATIONS-ONLY" METHODOLOGISTS

By overstating findings and publishing studies based on preliminary data, scientists are confusing the public and undermining valid research . . . Epidemiology is a crude and inexact science. Eighty percent of cases are almost all hypotheses. We tend to overstate findings, either because we want attention or grant money.

Williams L. Studies on diet add to confusion. *New York Times*. October 11, 1995.

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