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Dapsone Induced Hypohaptoglobinemia in Lepromatous Leprosy Patients[1]

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Intrinsic defects of some components of enzyme systems in erythrocytes render them more susceptible to the hemolytic actions of some drugs. Even mild intravascular hemolysis can lead to anemia. Hemolytic effects of dapsone in normal healthy whites and Negro men with a deficiency of glucose-6-phosphate dehydrogenase have been reported ([1,4]). Hemolysis is one of the most undesirable side effects in the treatment of leprosy patients with dapsone ([3]). Treatment of various dermatological disorders with varying doses of dapsone has resulted in hemolytic and cholestatic types of jaundice ([7,13,18]). The estimation of haptoglobin in serum has been of great clinical significance in assessing the extent of hemolysis ([8]). The present study was designed to determine the extent of hemolysis, if any, in leprosy patients by measuring the hemoglobin binding capacity of their sera.

MATERIALS AND METHODS

Twenty-two untreated lepromatous leprosy patients and 11 contacts of leprosy patients were selected as the subjects of the present study. All were males, ranging from 20 to 40 years in age. The healthy contacts of the patients were selected at random and served as controls for the present study. All the leprosy patients were admitted to the hospital ward and watched closely for a few days for any severe liver derangement and/or jaundice. Then they were put on dapsone therapy of 100 mg daily for 14 days. A period of 2 weeks of dapsone therapy was chosen arbitrarily. Fasting blood and urine samples were collected before and after 15 days of treatment. The sera were separated and analyzed immediately. Hemolysate was prepared by the method cited below and preserved at −20°C until use. A standard solution of hemoglobin containing 2.7 mg/0.02 ml was prepared by diluting the stock hemoglobin solution. Fresh standards were prepared for use every week.

Haptoglobins were estimated by the gel filtration technique of Ratcliff and Harwicks ([16]) cited by Harold Varley ([21]). To have a better correlation of the results, we also investigated simultaneously serum lactate dehydrogenase (LDH) ([21,p.279]), alkaline phosphatase ([21,p.455]), blood hemoglobin by the cyanomethemoglobin method ([21,p.585]), bilirubin ([21,p.353]), urinary excretion of urobilinogen ([21,p.366]) by the method of Watson, *et al.* and erythrocytes in urine by the conventional benzidine test. Statistical significance of the results was determined by applying student's T tests.

RESULTS

Haptoglobin concentration is expressed as milligrams of hemoglobin bound per 100 ml of serum. Control subjects showed a wide range of 86.6 ± 43.6 (mean ± S.D.) mg/100 ml serum. Untreated lepromatous cases recorded significantly higher ($p < 0.05$) levels of haptoglobin compared to controls. There was a remarkable and significant ($p < 0.05$) reduction in haptoglobin levels following treatment with dapsone. This could be correlated with a corresponding increase in serum bilirubin and urinary urobilinogen. There was a drop of nearly 2 grams in blood hemoglobin concentration following treatment ($p < 0.05$). Urinary urobilinogen was already elevated in untreated lepromatous cases, and more interesting is its increase with treatment. The benzidine test was negative in all of the urine samples studied. Alkaline phosphatase and lactate dehydrogenase values in-

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TABLE 1. *Blood/serum values in leprosy patients before and after 14 days' treatment with dapsone, 100 mg daily. Values are expressed as means ± S.D.*

Subjects	Number	Haptoglobin mg/dl	Bilirubin mg/dl	Hemoglobin grams/dl	Alkaline phosphatase I.U./l	LDH I.U./l
Controls	11	86.64 ± 10.49	0.90 ± 0.34	14.0 ± 0.6	60 ± 3	150 ± 55
Untreated lepromatous leprosy	22	109.69 ± 10.55	1.40 ± 0.54	12.2 ± 0.8	75 ± 12	200 ± 20
After dapsone therapy	22	67.09 ± 8.72	3.70 ± 0.96	10.2 ± 0.8	78 ± 23	255 ± 46

creased in leprosy patients following treatment, perhaps indicating some degree of liver damage. These results are presented in Tables 1 and 2.

DISCUSSION

The term "haptoglobin" was coined to designate a group of plasma proteins forming stable hemoglobin complexes in which the hemoglobin has acquired the properties of peroxidase ([12]). On electrophoresis they exhibit alpha-2 globulin mobility. There is no diurnal variation in the haptoglobin level in a person, but there may be a slight variation in the levels from person to person. This may be due to small differences in synthesis or due to differences in the amount of hemoglobin that enters the circulation every day. It is therefore understandable that a decrease in haptoglobin develops, often with only slightly increased red cell turnover, provided haptoglobin synthesis per se is not affected. Changes in haptoglobin are valuable aids in the diagnosis of hemolysis, especially mild intravascular hemolysis. As in other chronic infections associated with increased levels of haptoglobin ([11, 14]), elevated levels of haptoglobin were observed in all these untreated lepromatous patients. The increases in serum bilirubin and urinary urobilinogen accompanied by a significant drop in hemoglobin (2 grams/dl) after treatment with dapsone are indicative of a mild hemolysis. The significant drop in haptoglobin levels following dapsone therapy indicates a certain amount of hemolysis. Our results are in agreement with many other investigators who have reported that dapsone induces hemolysis ([5, 6, 9, 22]). Decreases in haptoglobin levels have been reported in different types of anemia such as pernicious anemia, hemolytic anemia (congenital and acquired), iron deficiency anemia and drug induced anemia, and in liver damage ([15]).

The normal haptoglobin pool is about 8 grams per 70 kg body weight. The interpretation of changes in haptoglobin levels is somewhat more complicated than those of other plasma proteins since no absolute relationship exists between the blood haptoglobin level and synthesis. An elevated haptoglobin level can be attributed to renal dysfunction ([10]). We have observed a significant degree of renal dysfunction in untreated lepromatous patients ([17]). Steroids are known to elevate serum haptoglobin levels ([2, 19, 20]). It is rather interesting that despite the reported subnormal functioning of the adrenal cortex in leprosy patients ([2]) the haptoglobin levels remain high in untreated lepromatous cases. The decrease in haptoglobin levels following treatment with dapsone could be due to an increased rate of catabolism of the haptoglobin-hemoglobin complex by the reticuloendothelial sytem without any further

TABLE 2. *Urinary findings in leprosy patients before and after 14 days' treatment with dapsone, 100 mg daily. Values are expressed as means + S.D.*

Subjects	Number	Urobilinogen mg/dl	Benzidine test
Controls	11	1.28 ± 0.52	–
Untreated lepromatous leprosy	22	5.16 ± 0.45	–
After dapsone therapy	22	9.63 ± 0.83	–

compensatory increase in haptoglobin synthesis.

Another possibility is that the decreased haptoglobin levels could be a mechanism to conserve iron and could be compensatory to circumvent the intrinsic hemolytic tendency. Dapsone has been found to alter the structural integrity and quantity of components ([5]) essential for keeping the erythrocytes intact in the presence or absence of a hemolytic agent. Dapsone has been reported to induce low levels of glutathione with simultaneous deficiencies of catalase and ATPase in erythrocytes ([6]). These changes could account for the hemolysis and anemia observed in leprosy patients following dapsone therapy.

SUMMARY

Dapsone has been used in various dermatological disorders and in leprosy. One of the main side effects of dapsone therapy is anemia, mostly hemolytic. We aimed at finding the effect of dapsone therapy on serum haptoglobin levels which could be an indirect evidence for intravascular hemolysis, supported by secondary investigations such as liver functions (serum lactic dehydrogenase, alkaline phosphatase, bilirubin), blood hemoglobin levels, urinary excretion of urobilinogen, and erythrocytes. As in other infectious conditions, haptoglobins were raised in untreated lepromatous cases, compared to controls ($p < 0.05$). Dapsone treatment of 100 mg daily for 14 days brought down the haptoglobin level significantly as compared to the untreated cases and the controls ($p < 0.05$). An elevated alkaline phosphatase and lactate dehydrogenase indicate some liver dysfunction following dapsone therapy. A significant drop in blood hemoglobin level and a concomitant increase in serum bilirubin, urinary excretion of urobilinogen, and a significant fall in the serum hemoglobin binding capacity (haptoglobin level) following treatment with dapsone are quite suggestive of mild intravascular hemolysis.

RESUMEN

La dapsona se ha usado en varios desórdenes dermatológicos y en la lepra. Uno de los principales efectos colaterales de la terápia con dapsona es la anemia, especialmente la hemolítica. En este trabajo se decidió investigar el efecto de la terápia con dapsona sobre los niveles de haptoglobina sérica. Esto, junto con los datos sobre la función hepática (deshidrogenasa láctica, fosfatasa alcalina, bilirrubina), los niveles de hemoglobina en sangre, la excreción urinaria del urobilinógeno, y las cuentas de eritrocitos, podría ayudar a establecer la ocurrencia de hemólisis intravascular. Al igual que en otras condiciones infecciosas, las haptoglobinas se encontraron elevadas en los casos lepromatosos no tratados, en comparación con los controles ($p < 0.05$). El tratamiento con 100 mg diarios de dapsona durante 14 días, abatió el nivel de haptoglobina de manera muy significativa en comparación con los casos no tratados y con los controles ($p < 0.05$). La elevación en los niveles de fosfatasa alcalina y de lactato deshidrogenasa, indicó cierta disfunción hepática subsecuente a la terápia con dapsona. La importante caída en el nivel de hemoglobina sanguínea y el concomitante incremento en la bilirrubina sérica y en la excreción urinaria de urobilinógeno, así como la caída en la capacidad de captación de hemoglobina sérica (nivel de haptoglobina) después del tratamiento con dapsona, son muy sugestivos de la ocurrencia de hemólisis intravascular moderada.

RÉSUMÉ

La dapsone a été utilisée pour le traitement de différents troubles dermatologiques, de même que pour la lèpre. Un des effets secondaires principaux de la thérapeutique par la dapsone est l'anémie, surtout hémolytique. On a tenté de rechercher l'effet de la thérapeutique de la dapsone sur les niveaux d'haptoglobines sériques, ce qui pourrait constituer une indication indirecte d'une hémolyse intravasculaire, ainsi qu'il est suggéré par des explorations secondaires menées sur la fonction hépatique (déhydrogénase lactique du sérum, phosphatase alcaline, bilirubine), sur les niveaux d'hémoglobines du sang, sur l'excrétion urinaire de l'urobilinogène, et sur les érythrocytes. Ainsi que c'est le cas dans d'autres infections, les haptoglobines étaient élevées dans les cas de lèpre lépromateuse non traités, par rapport aux valeurs trouvées chez les témoins ($p < 0.05$). Le traitement par la dapsone, à raison de 100 mg par jour, pendant 14 jours, a entraîné une dimunution significative des taux d'haptoglobines, comparé à ce que l'on a constaté chez les cas non traités et chez les témoins ($p < 0.05$). Des taux élevés de phosphatase alcaline et de lactate déhydrogénase signalent un trouble de fonctionnement du foie faisant suite à la thérapeutique par la dapsone. Une chute significative dans les taux d'hémoglobines du sang, de même qu'une augmentation simultanée de la bilirubine du sérum et de l'excrétion urinaire de l'urobilinogène, accompagnés d'une diminution significative de la capacité de liaison de l'hémoglobine du sérum (taux d'haptoglobine), toutes cette observations faites à la suite du traitement par la dapsone, suggèrent une hémolyse intravasculaire légère.

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