A Pharmacological Study of a New Sulfonamide In Glucose-6-Phosphate Dehydrogenase Deficient Subjects

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SULFACYTINE (1-ethyl-N-sulfanilylcytosine) has recently been described by Doub and associates[1] as a new, highly soluble sulfonamide with the empirical formula of $C_{12}H_{14}N_4O_3S$. The half-life is approximately 4 hours. Sulfacytine enters the urine as almost entirely free, or microbiologically active, drug, is less protein-bound than sulfisoxazole, and demonstrates two to three times the bacteriostatic activity of sulfisoxazole when studied in experimentally-induced animal infections.[2] Sulfacytine should be clinically effective in managing urinary tract infections due to susceptible microorganisms at a lower dose than sulfisoxazole.

Sulfonamides belong to the group of oxidizing drugs that are capable of inducing hemolysis in patients deficient in glucose-6-phosphate dehydrogenase (G-6-PD).[3] Complex glucose metabolic processes occur in the erythrocyte as energy sources.[4] G-6-PD is necessary to maintain an adequate concentration of reduced glutathione, which in turn prevents the reduction of hemoglobin to methemoglobin. Patients deficient in G-6-PD will have depressed levels of glutathione. The presence of an oxidizing drug may further interfere with the regeneration of glutathione. Hemoglobin is then more easily reduced to methemoglobin and hemolysis of the erythrocyte can result.[3]

The reported incidence of G-6-PD deficiency is from 10 to 15 per cent in Negroes, about 1 per cent in Caucasians.[5] Although Negroes have a higher incidence, the pathologic process appears to be milder and self-limiting. Since sulfacytine must be considered an oxidizing sulfonamide, a study in G-6-PD deficient patients was performed and is the subject of this report. The dose of sulfacytine selected for study was 2.0 grams/day. This is twice the chemically effective dose of sulfacytine (1.0 gram/day). The dose selected for sulfisoxazole was 4.0 grams/day, the recommended therapeutic dose of sulfisoxazole.

Materials and Method

Fourteen known G-6-PD deficient Negro volunteers participated in the study. G-6-PD deficiency was confirmed in each participant before starting the trial by determining G-6-PD activity,[6] by glutathione assay,[7] and by methemoglobin reduction.[8] Ten normal Caucasian males also participated as controls (non-G-6-PD deficient). All were inmates at the State Prison of Southern Michigan, Jackson, Michigan. Written informed consent was obtained by one of us (TCS).

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A physical examination, blood count, and chemical laboratory determinations were performed before the trial to rule out any metabolic defects which might interfere with sulfonamide metabolism. If any of the participants had recently suffered a hemolytic crisis, they might not have the capability to do so again during this investigation. The glutathione instability test was therefore performed on each participant just before the trial to assess this capability.[9]

The 14 G-6-PD deficient participants were randomly assigned to one of three treatment groups: *(1)* sulfacytine 2.0 grams loading, then 2.0 grams per day for 14 days, *(2)* sulfisoxazole 4.0 grams loading, then 4.0 grams per day for 14 days, or *(3)* placebo. The ten normal or control subjects randomly received either sulfacytine at the same dose or placebo. All medication was divided into four daily doses (7 A.M., 11 A.M., 3 P.M., and 7 P.M.).

The following studies were performed daily throughout the study and for five days following the termination of medication: hemoglobin, hematocrit, red cell count, reticulocyte count, bilirubin, urinalysis (for occult blood and urobilinogen), glutathione, and methemoglobin. All laboratory results were reported to the principal investigator immediately so that any evidence of hemolysis would be promptly detected.

Results

The first indication of an impending hemolytic crisis in a G-6-PD deficient patient would be a further reduction in the glutathione level and subsequent elevation of the methemoglobin concentration. The mean glutathione values are displayed in Fig. 1 and the mean methemoglobin values in Fig. 2 for the three different regimens administered to the G-6-PD deficient participants.

The values for the control group are not included in order to maintain simplicity in the illustrations. The pretreatment glutathione levels in the control ranged from 68 to 81 mg/100 ml. These are greater than the pretreatment levels in the deficient group (Fig. 1), as would be expected. The pretreatment methemoglobin values for the controls ranged from 0.03 to 0.13 Gm/100 ml.

Glutathione dropped slightly on the second day, then returned to the previous level. The methemoglobin values demonstrate an increase on the third day, with the greatest increase being in the placebo group. The values then fall promptly back to the pretreatment level.

Had hemolysis occurred, the reticulocyte count would be expected to rise, the hematocrit to fall, and the bilirubin to rise. These parameters are displayed in Figs. 3, 4, and 5 for the three regimens. The reticulocyte counts demonstrate a rise on day 6, the same elevation occurring in the placebo group. The counts then fall back to their previous values. No particular changes are noted in the hematocrit or bilirubin values.

The glutathione instability test was normal prior to the start, indicating that all were capable of hemolysis.

Discussion

Neither the predictive tests for hemolysis (glutathione and methemoglobin) nor those that reflect hemolysis (reticulocyte count, hematocrit, and bilirubin) demonstrated any obvious evidence of hemolysis in this study. Sulfisoxazole was administered at the usual therapeutic dose while sulfacytine was given at twice the clinically effective dose. Neither sulfacytine nor sulfisoxazole appeared to induce hemolysis under the conditions of this trial.

G-6-PD deficiency is more common in the Negro population but tends to be more benign in Negroes than in Caucasians. Caucasians with this deficiency

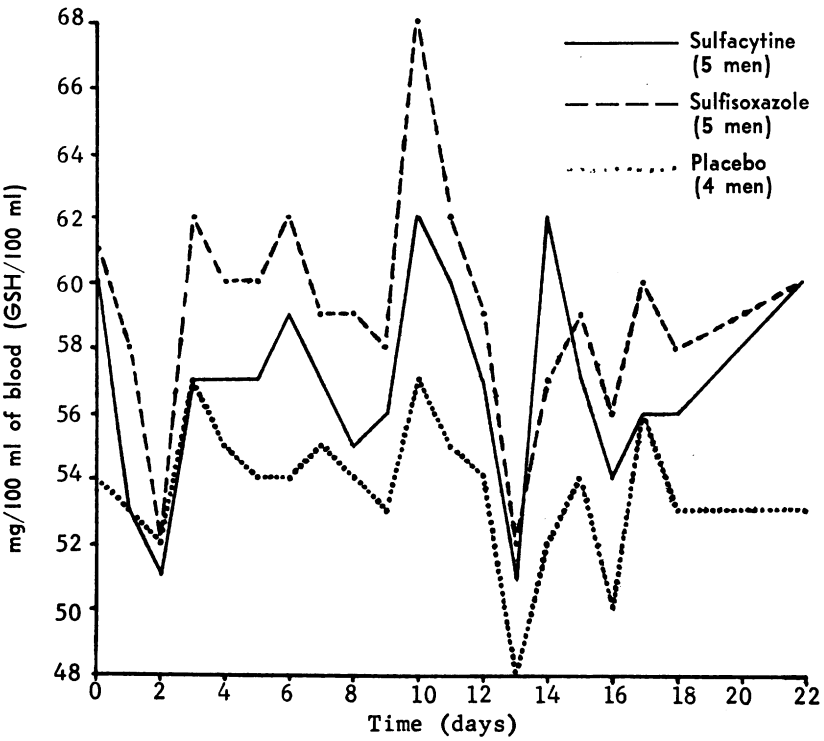

Fig. 1. Mean glutathione values in G-6-PD deficient subjects.

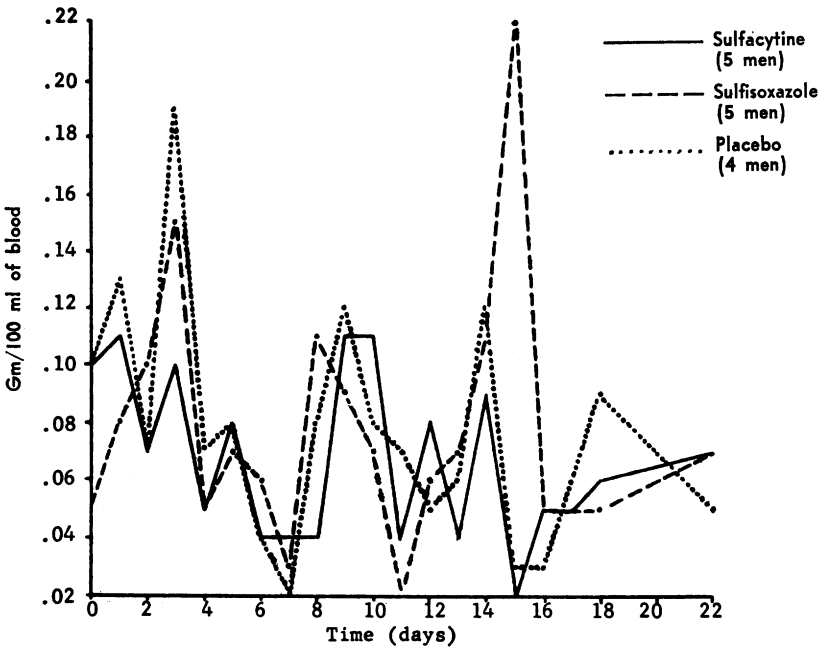

Fig. 2. Mean methemoglobin values in G-6-PD deficient subjects.

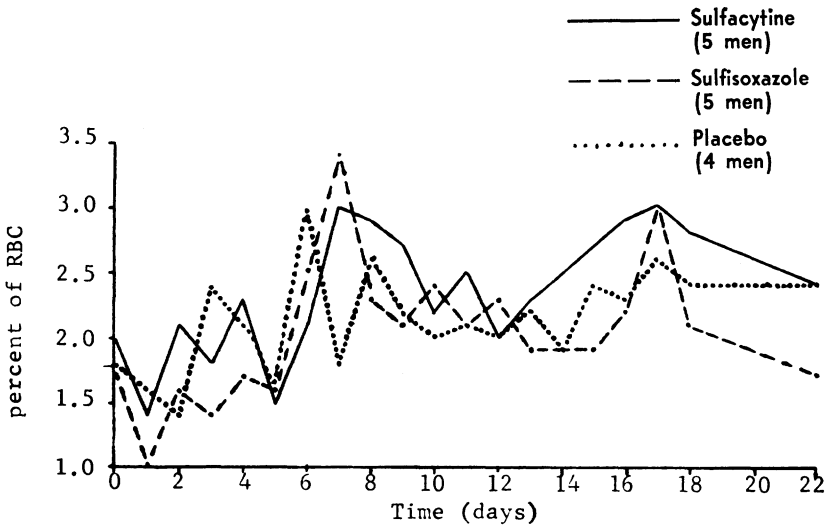

Fig. 3. Mean reticulocyte count in G-6-PD deficient subjects.

were specifically excluded for fear of inducing a catastrophic hemolytic episode.

In addition to providing information about sulfacytine, this investigative procedure might be useful in studying other new oxidizing drugs. Adequate controls including either a placebo or reference compound should be used. Both predictive

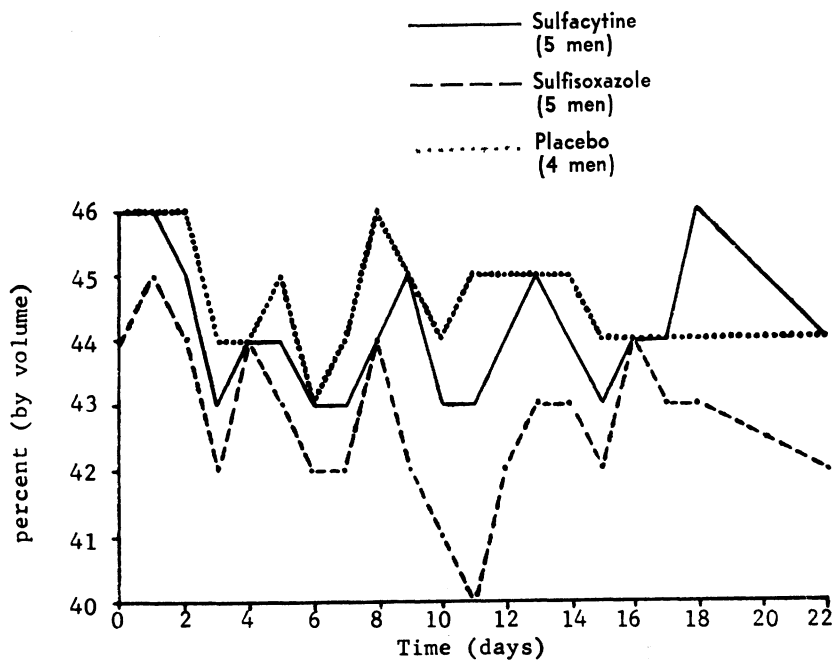

Fig. 4. Mean hematocrit in G-6-PD deficient subjects.

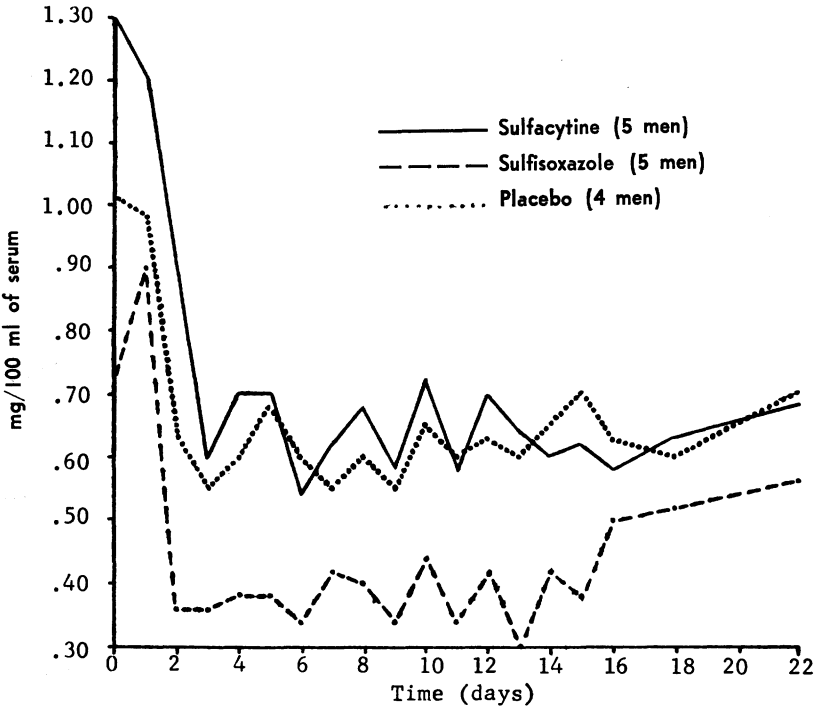

Fig. 5. Mean bilirubin values in G-6-PD deficient subjects.

studies and those reflecting hemolysis are useful. Flatz et al. performed a similar trial with sulfalene (sulfamethoxypyrazine) and failed to induce hemolysis.[10]

Summary

Neither sulfacytine nor sulfisoxazole induced erythrocyte hemolysis when given to G-6-PD deficient Negroes under the conditions of this study. The sulfacytine dose was twice the therapeutic dose.

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