In Vitro Effect of Dapsone on NADH-Methemoglobin Reductase

TO THE EDITOR:

Leprosy patients under sulfone therapy exhibit both a wide variability and a raised NADH-methemoglobin reductase activity ([3]). The enzyme activity is also negatively correlated to the patients' hemoglobin level ([3]) but not to their reticulocyte rate ([2]). Considering that NADH-methemoglobin reductase (actually a NADH-cytochrome b_5 reductase) is a membrane enzyme ([6]) and that dapsone exerts significant hemolysis ([1]) due to its property to oxidize membrane lipid and protein components ([4]), one possible hypothesis to explain the rise in enzyme activity is that the action of dapsone upon the cell membrane could somehow augment the soluble fraction of NADH-methemoglobin reductase. According to this hypothesis, both the great variability and the rise in enzyme activity might be explained.

We carried out experiments on blood samples obtained from 14 volunteers. After washing the red cells with an isotonic-buffered saline solution, each sample was suspended in the same medium at approximately 50% hematocrit and divided into six portions of 1 ml each. Five portions received 2–10 μg dapsone/ml erythrocyte suspension; the other portion received none. They were incubated for 2 hr at 37°C with gentle stirring. After incubation, NADH-methemoglobin reductase activity was determined according to Scott ([5]) in both the crude hemolysate and unsealed ghosts, the latter prepared according to Steck and Kant ([7]). Scott's method ([5]) is based on a dye reduction (dichloro-indophenol, DCIP) by NADH in the presence of either crude hemolysate or cell membrane suspension after treatment of the erythrocytes with 1% $NaNO_2$ in isotonic-buffered saline followed by additional washing. Enzyme activity was expressed as (ΔA_{600}/min per mg protein) × 10^4.

We also measured the enzyme activity by applying the same method ([5]) to the supernatant of erythrocyte ghosts which were not previously treated with dapsone, before and after the addition of 5 μg dapsone/ml membrane suspension. A period of incubation of 10 min at room temperature was followed by another centrifugation for 15 min at 22,000 × *g*. The enzyme reactions in both cases proceeded for 5 min, and the results were expressed as $\Delta A_{600} \times 10^3$.

For the erythrocytes previously incubated with dapsone, we found a dose-dependent increase of NADH-methemoglobin reductase activity measured in the crude hemolysate ($r = 0.40$; $N = 36$; $p < 0.05$) and a negative correlation between this variable

and the enzyme activity measured in membrane suspension prepared from the same portion ($r = -0.38$; $N = 29$; $p < 0.05$). One likely interpretation is that dapsone promotes the increase in enzyme activity in the hemolysate by releasing enzyme molecules attached to the cell membrane.

Data concerning the eight determinations of NADH-methemoglobin reductase activity in the supernatant of ghost suspension, before and after the treatment with dapsone, strongly supports this interpretation. The mean enzyme activity before the incubation with dapsone was 9.13 units (S.D. = 2.17). After the incubation with the drug, the mean value jumped to 42.00 units (S.D. = 18.38), the difference being highly significant as shown by paired observation analysis ($t = 5.53$; $DF = 7$; $p < 0.01$). It is also noteworthy that the coefficient of variation increased from 23.75% to 43.77%, which is in accordance with the great variance observed in the activity of NADH-methemoglobin reductase from leprosy patients under sulfone therapy.

The present data may also explain the observed negative correlation between the hemoglobin level and the enzyme activity in leprosy patients ([3]), since both effects are dependent on dapsone concentration.

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