Studies on the Mechanism of Chloramphenicol Impairment of Human Leukocyte Function[1] (34778)

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This study attempts to define the mechanism of chloramphenicol impairment of the respiratory burst of polymorphonuclear (PMN) leukocytes subsequent to particle ingestion (1). Recent studies have shown that the intracellular killing of ingested bacteria is closely linked with this respiratory burst (2–4). Clinical substantiation of this relationship is found in patients with granulomatous disease (5) who demonstrate both a propensity for frequent bacterial infections and impaired PMN leukocyte respiratory augmentation after phagocytosis (6). In this study, the major parameters evaluated in the chloramphenicol-exposed PMN leukocytes were oxygen consumption, hexose monophosphate (HMP) shunt activity, and NADH oxidase activity of both resting and phagocytizing leukocytes.

Materials and Methods. Leukocyte-rich plasma was obtained by allowing heparinized whole blood to settle at room temperature for 30–60 min. Oxygen consumption and phagocytosis studies with chloramphenicol were performed as previously described (1) using 1 ml of WBC-rich plasma containing 1–3 $\times$ 10^7 leukocytes plus 2 ml of modified Krebs-Ringer phosphate buffer, pH 7.4. In four sets of experiments, 5 $\times$ 10^{-3} *M* NAD (P.L. Biochemicals) was added to duplicate flasks with and without chloramphenicol (2 $\times$ 10^{-3} *M*) just prior to attaching the flasks to the manometer. HMP shunt activity during phagocytosis was measured through the evolution of $^{14}CO_2$ from the metabolism of 1-glucose ^{14}C. The procedure consisted of the addition of 0.25 μCu of the labeled glucose from a side arm along with latex particles (Dow Chemical Company Lot LS-1165-B). One hour after the addition of latex, the experiment was terminated, and the radioactivity was determined using a Nuclear Chicago 720 liquid scintillation counter. The effect of chloramphenicol and of NAD plus chloramphenicol on cells was expressed as a percentage of the control where a value of 100% was assigned for the oxygen consumption or HMP shunt activity without chloramphenicol or NAD.

Leukocyte oxidase activity was evaluated by means of nitroblue tetrazolium (NBT) reduction. Leukocyte-rich plasma was centrifuged at 900 rpm, washed twice with Krebs–Ringer phosphate buffer, and suspended in Krebs–Ringer phosphate buffer containing 20% autologous plasma. The final total granulocyte count was in the range of 2–3 $\times$ 10^7 cells. Half of the cells then were incubated with 6 $\times$ 10^{-3} *M* chloramphenicol and half with saline for 90 min. One-twentieth milliliter of a 0.1% solution of NBT in buffered saline at pH 5 was pipetted into each of 12 tubes (10 ml) containing 1 m*M* of potassium cyanide. One twentieth milliliter of latex particles (0.79μ) were added to half of the tubes and 0.05 ml of buffer to the others. A 0.5-ml portion of the leukocyte suspensions previously incubated with and without chloramphenicol then were added to NBT containing tubes, in triplicate, with and without latex. The tubes were shaken several times and were placed in a 37° water bath and incubated for 15 min with manual shaking every 4–5 min. The blue formazan then was extracted by the addition of 4.0 ml of chloroform:absolute methanol (1:1/v:v). The tubes were stoppered, mixed on a vortex

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mixer, and then centrifuged for 5 min at 2000 rpm. The chloroform layer was removed with a Pasteur pipette, and the optical density of the blue color was determined on a Hitachi Perkin-Elmer spectrophotometer at 510 mμ against a reagent blank. The effect of chloramphenicol was analyzed by means of a paired *t* test.

Results. The effect of varying amounts of chloramphenicol and of chloramphenicol plus NAD on leukocyte oxygen consumption, HMP shunt activity, and phagocytosis are shown in Fig. 1. Resting leukocyte respiration was slightly to moderately diminished in each of the three experiments at 6×10^{-3} *M* chloramphenicol and in five of the nine experiments when 2×10^{-3} *M* was used. Inhibition of resting respiration was partially reversed in the presence of 2×10^{-3} *M* chloramphenicol in two of four instances after the addition of NAD. Chloramphenicol in a concentration of 2×10^{-4} *M* did not significantly decrease resting respiration. Chloramphenicol inhibition of phagocytizing respiration and HMP shunt activity followed nearly identical patterns. Despite severe inhibition of phagocytizing respiration and HMP shunt activity at 6×10^{-3} *M* chloramphenicol, some phagocytosis was seen. In one of the three experiments at this concentration, 68% of the PMN leukocytes contained 30 or more particles. With 2×10^{-3} *M* chloramphenicol, phagocytizing respiration and HMP shunt activity were impaired ($p = < .01$) in nine of nine experiments with mean values of 36% and 40% of control values, respectively. The addition of NAD partially reversed the chloramphenicol inhibition of phagocytizing respiration and HMP shunt activity in all studies, such that the mean phagocytizing respiration and HMP shunt activity values were improved to 65% and 62% of control values, respectively.

Phagocytosis was not impaired in the presence of 2×10^{-3} *M* chloramphenicol. No significant changes in phagocytosis, phagocytizing respiration, or HMP shunt activity were observed with 2×10^{-4} *M* chloramphenicol.

The results of studies with NBT are indicated in Fig. 2. Leukocytes preincubated with 6×10^{-3} *M* chloramphenicol showed a marked decrease in NBT reduction both in the resting and the phagocytizing states

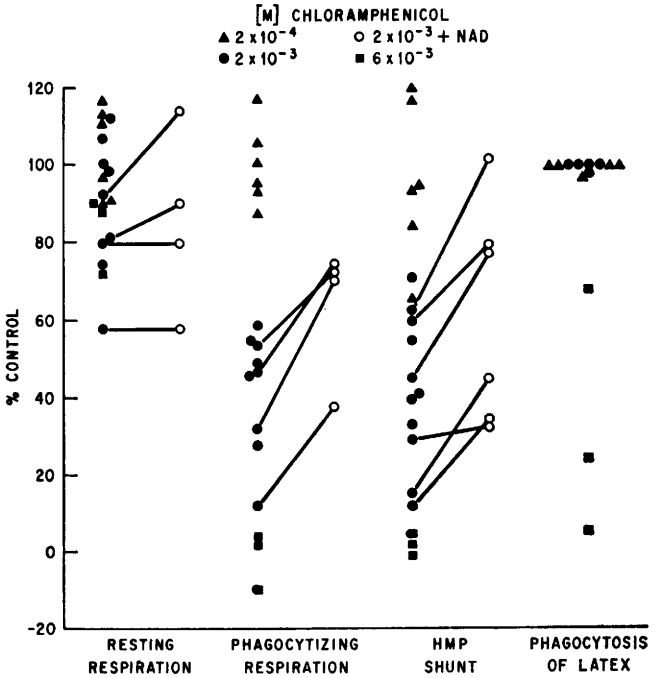

FIG. 1. Effect of chloramphenicol and NAD on some aspects of PMN leukocyte function.

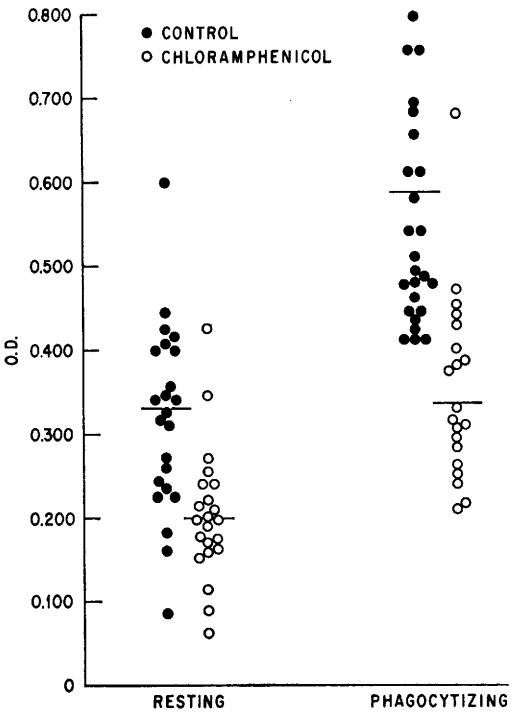

FIG. 2. Effect of chloramphenicol on NBT reduction in resting and phagocytizing PMN leukocytes.

($p = < .01$ in both instances). Without chloramphenicol the mean resting OD was 0.330 and the phagocytizing OD was 0.585 while with chloramphenicol the resting and phagocytizing values were 0.200 and 0.335, respectively, with the Δ OD being 53% of the control in the presence of chloramphenicol.

Discussion. Simultaneous measurements of oxygen consumption and HMP shunt activity in PMN leukocytes in the presence of chloramphenicol demonstrate that the inhibition of the respiratory burst associated with phagocytosis is directly related to its effect on HMP shunt activity. Nearly identical degrees of impairment of these two parameters were observed (Fig. 1).

Previous studies in this laboratory using whole blood demonstrated an inhibition by chloramphenicol of the usual burst in oxygen consumption associated with phagocytosis (1). This inhibition was reversed by methylene blue suggesting that the HMP shunt was intact and that the problem was one of impairment of shunt activation. It has been suggested that similar effects could be the result of defective NADPH or NADH oxidase (7, 8).

While the amount of available NADP is rate-limiting for the HMP shunt (9), the means by which NADP is generated in the cell remains controversial (8, 10–14). A large body of evidence suggests that NADP may be generated in the cell by means of a cyanide-insensitive NADPH oxidase, activated with phagocytosis of particles. Other investigators, however, finding a cyanide-insensitive NADH oxidase and a cyanide-sensitive NADPH oxidase propose an indirect pathway through an NADPH-linked lactic dehydrogenase (10). The generation of NADP from this step would then activate the HMP shunt. If nitroblue tetrazolium reduction can be used as an index of NADH oxidase activity (15), then the impaired NBT reduction may be interpreted as partial inhibition of this enzyme by chloramphenicol. However, nitroblue tetrazolium reduction probably is not specific since the hydrogen ion for its reduction may be available from other sources such as NADPH oxidase. Chloramphenicol, therefore, could interfere totally with one means of NBT reduction or partially with several. If NADH oxidase were specifically inhibited by chloramphenicol, we might expect correction of the impaired respiratory burst by the addition of NAD and we indeed observed a partial correction. Several experiments using higher concentrations of NAD failed to increase this degree of correction.

The hypothesis that chloramphenicol inhibits nucleotide oxidases is supported by the observation of Freeman and Halder who used a beef heart mitochondrial system (16). They found that 6×10^{-3} *M* chloramphenicol almost completely inhibited the oxidation of NADH and that 10^{-3} *M* chloramphenicol was associated with a 50% inhibition of NADH oxidation. The degree of inhibition with these concentrations of chloramphenicol closely agrees with the degree of respiratory and HMP shunt inhibition in our preparations, and with the degree of respiratory inhibition of leukocyte homogenates shown by Follett *et al.* (17).

Our data and those of Freeman and Halder

suggest that chloramphenicol partially inhibits oxidation of NADH, which, in turn, is associated with partial impairment of HMP shunt activation. This is best demonstrated in human leuocytes with a 2×10^{-3} *M* concentration of chloramphenicol. The fact that correction of the chloramphenicol-induced functional impairment was only partial with NAD while complete with methylene blue may be due to the known poor permeability of cells to nucleotides. However, the possibility that other enzymes may be involved has not been excluded. Because of the high concentration of chloramphenicol used in this experiment, it is difficult to implicate this toxic effect on leukocytes as a mechanism of clinical toxicity. However, chloramphenicol is cleared more slowly from the blood of patients demonstrating hematologic toxicity (18), and the possibility of selective concentration by cells has not been excluded. It would be of interest to learn whether intracellular killing of ingested organisms is impaired using these concentrations of chloramphenicol.

Summary. Chloramphenicol modification of the phagocytosis-induced respiratory burst of human PMN leukocytes may be directly correlated to impairment of hexose monophosphate shunt activity. Evidence is presented that impairment of HMP shunt activity may be due to lack of activation of the shunt through partial inhibition of NADH oxidase.

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