ROLE OF OXIDANTS IN THE MICROBICIDAL MECHANISMS OF POLYMORPHONUCLEAR LEUKOCYTES

PART 1

EFFECT OF HYDROGEN PEROXIDE AND SUPEROXIDE RADICAL ON VIABILITY OF *NEISSERIA GONORRHOEAE* AND RELATED BACTERIA

PART 2

IMPROVEMENT OF POLYMORPHONUCLEAR LEUKOCYTE OXIDATIVE AND BACTERICIDAL FUNCTIONS IN CHRONIC GRANULOMATOUS DISEASE WITH 4-AMINO-4'-HYDROXYLAMINODIPHENYL SULFONE

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ABSTRACTS

Title: Role of oxidants in the microbicidal mechanisms of polymorphonuclear leukocytes.

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Part 1: Effect of hydrogen peroxide and superoxide radical on viability of Neisseria gonorrhoeae and related bacteria.

The oxidative killing of bacteria within polymorphonuclear leukocytes (PMN) is thought to involve the generation of hydrogen peroxide (H_2O_2), superoxide anion (O_2^-) singlet oxygen ($^1O_2$) and the hydroxyl radical ($OH^\cdot$). The sensitivity of bacteria to these agents can be investigated in vitro by incubation of the organisms with purine and xanthine oxidase (XO). The oxidation of purine by XO generates O_2^- which spontaneously dismutates to H_2O_2, $^1O_2$ and $OH^\cdot$. The sensitivity of Neisseria to the products of XO reaction was determined. N. gonorrhoeae, N. meningitidis, N. perflava and Branhamella catarrhalis are killed by the XO reaction products; and H_2O_2,but not O_2^- or $OH^\cdot$,appears to be the bactericidal agent. This conclusion is based on the ability of catalase to inhibit killing whereas the O_2^--scavenger, superoxide dismutase (SOD) slightly enhances killing. Mannitol, a scavenger for $OH^\cdot$, shows no effect. That H_2O_2 is the oxidant responsible for gonococcal killing is confirmed by the observed sensitivity of gonococci in the glucose-glucose oxidase system, a reaction

that generates H_2O_2 directly. Previous studies indicate that virulent colony-type 1 gonococci are more resistant to phagocytosis by PMN than are avirulent colony-type 4 gonococci, and that once ingested, gonococci are killed. Utilizing the purine-XO bactericidal system, gonococci type 1 and 4 are found to be similar in their sensitivity to the products generated in the system. Both exponential and stationary phase gonococci are killed to the same degree in the XO system, and the presence of lactoperoxidase plus iodide in the system only slightly, if at all, enhances killing of gonococci. Together, these results tend to support the view that gonococcal virulence is attributed to their ability to resist phagocytosis by PMN and that if ingested, gonococci will succumb to the leukocytic bactericidal mechanisms.

Part 2: Improvement of polymorphonuclear leukocyte oxidative and bactericidal functions in chronic granulomatous disease with 4-amino-4'-hydroxylaminodiphenyl sulfone.

Chronic granulomatous disease (CGD) leukocytes fail to exhibit the metabolic activities normally associated with phagocytosis, which include stimulation of hexose monophosphate (HMP) shunt activity, iodination of ingested protein and the production of H_2O_2 and O_2^-. The pathologic basis for the disorder is diminished H_2O_2 production by CGD PMN which leads to a failure to kill catalase-positive organisms. In an effort to restore oxidant-dependent capabilities of these cells, the in vitro effect of a dapsone derivative, 4-amino-4'-hydroxylaminodiphenyl sulfone (DDS-NOH), on CGD PMN functions was studied. DDS-NOH has been previously shown to produce H_2O_2 intracellularly. After incubation of CGD PMN with 0.2 or 1.0 mM DDS-NOH for 30 min, the rate of glucose-1-^{14}C oxidation via HMP shunt increases 2-4 fold above normal values. Similar concentrations of DDS-NOH also enhance iodination of ingested zymosan particles 1.5 - 2.7 fold above resting values. Both metabolic effects can be further enhanced by SOD but inhibited by catalase, indicating that O_2^- is an intermediate product generated by DDS-NOH and that the correction of CGD PMN metabolic activities is H_2O_2-mediated. In the presence of 0.2 mM DDS-NOH, improved in vitro killing of Staphylococcus aureus is observed with PMN from three patients suffering CGD. DDS-NOH at 0.2 mM concentration has no effect on phagocytosis of C3-coated-lipopolysaccharide-paraffin oil droplets by CGD PMN but was inhibitory for normal PMN. However, at 1.0 mM, DDS-NOH inhibits phagocytosis by both CGD and normal PMN. At concentrations as low as 0.02 mM, DDS-NOH induce capping of Concanavalin A receptor complexes, suggesting interference by the oxidant drug with microtubule-associated function. Thus, optimal concentration of DDS-NOH may be employed as an oxidant to improve metabolic and bactericidal functions of PMN from patient with CGD.