Candidacidal Activity of Myeloperoxidase: Therapeutic Influence of the Enzyme In Vivo

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Mice were injected intravenously with Candida albicans blastospores to establish chronic renal infection. Seventy-five percent of the animals inoculated with 10⁶ blastospores died as a consequence of infection during the subsequent 60 days of observation. Intraperitoneal administration of 10 mU of partially purified human myeloperoxidase 1 day after injection of the pathogen increased survival of the mice from 25 to 80% over this time period. Administration of myeloperoxidase complexed with soluble C. albicans cell wall mannan abrogated the protective influence of the enzyme. These results demonstrate that exogenous myeloperoxidase has a therapeutic influence on murine renal candidiasis and suggest that exogenous myeloperoxidase may also be effective in the treatment of certain forms of candidiasis in humans. These results also demonstrate the important role of the mannan-binding function of myeloperoxidase for effective treatment of candidiasis and suggest a mechanism of inhibition of the candidacidal effect of free enzyme in vivo by mannan accumulating in tissue fluids.

Myeloperoxidase is a major contributor to the microbicidal activity of neutrophils (5, 6). The microbicidal activity of myeloperoxidase depends upon two properties of the enzyme. An ability of myeloperoxidase to catalyze the reaction of hydrogen peroxide and chloride to generate the potent oxidant hypochlorous acid is essential for its microbicidal effect (1). An ability of the enzyme to bind to target microorganisms also contributes significantly to the microbicidal activity of myeloperoxidase. The importance of this target-binding function to myeloperoxidase-mediated killing of microorganisms has been demonstrated for both bacterial (10) and fungal (11) pathogens in vitro. The increased microbicidal activity of the target-bound enzyme most likely results from generation of hypochlorous acid in close physical proximity to the microbial substrates.

Okuda et al. (8) have suggested that lactoperoxidase might be useful for the treatment of cancer and infectious disease by complexing the enzyme with antibody specific for the desired target. Using a murine model of renal candidiasis, they demonstrated a significant therapeutic effect of lactoperoxidase conjugated to anti-Candida antibody. Based upon our observation that human myeloperoxidase has an innate ability to bind to Candida albicans (12, 13), we have tested the possibility that administration of myeloperoxidase alone to mice with chronic renal candidiasis (7) might have a therapeutic influence like that described for the lactoperoxidase-antibody conjugate. By comparing the therapeutic effects of myeloperoxidase and myeloperoxidase complexed with soluble C. albicans cell wall mannan, we have also considered the importance of the target-binding function of the enzyme to its candidacidal activity in vivo.

MATERIALS AND METHODS

Isolation of myeloperoxidase. Human neutrophils were isolated from blood drawn from healthy volunteers as previously described (10). Myeloperoxidase was isolated from the purified neutrophils by the method of Andrews and Krinsky (2), which essentially involved the following steps. The neutrophils were lysed by the addition of cetrimethylammonium bromide, and the cellular debris was removed by centrifugation. Myeloperoxidase was precipitated by the addition of ammonium sulfate, the salt was removed by dialysis against distilled water, and the enzyme was chromatographed on a G-50 Sephadex column in distilled water. Material in the 430-nm absorption peak was then rechromatographed on a Bio-Gel A-5M column (Bio-Rad Laboratories) in Tris-cetrimethylammonium bromide. Salts were removed by extensive dialysis against distilled water. The reinheit zah (R.Z.) value of the final peroxidase-containing fraction was 0.62 (2). Myeloperoxidase activity was quantitated in terms of pyrogallol units as described by Baggioni et al. (3).

The candidacidal activity of the enzyme preparation was characterized in vitro. Candidacidal activity was shown to be both hydrogen peroxide and chloride dependent. Inhibitors of myeloperoxidase activity (0.1 mM azide, 0.1 mM cyanide, and 1 mM L-methionine) also effectively inhibited the killing of target C. albicans (11).

Isolation of mannan. C. albicans 2252 (ATC 44806) was grown to the stationary phase in yeast nitrogen base minimal medium (4). Mannan was isolated with Fehling solution as copper complexes from citrate-extracted yeast as described by Peat et al. (9).

Test of therapeutic effect of myeloperoxidase in murine renal candidiasis. Renal candidiasis was established in 6- to 8-week-old male Swiss Webster mice by injection into the tail vein of 10⁵ to 10⁶ C. albicans 2252 blastospores in a 0.5-ml volume of phosphate-buffered saline. C. albicans concentrations were determined by pour plate methodology with yeast extract-peptone-glucose agar (4). The mortality of the C. albicans-infected mice was monitored over the subsequent 60-day period. One day after injection of the C. albicans, each mouse received an intraperitoneal injection of 0.5 ml of phosphate-buffered saline or phosphate-buffered saline containing 10 mU of myeloperoxidase, 20 mg of mannan, or a mixture of myeloperoxidase and mannan.

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Our results also demonstrate an important role for the mannan-binding function of myeloperoxidase for effective treatment of candidiasis in this model and suggest a mechanism of inhibition of the candidacidal effect of free enzyme in vivo by mannan accumulating in tissue fluids.

In the murine model of chronic renal candidiasis, the therapeutic effect of myeloperoxidase in this study was achieved, however, without conjugation of the myeloperoxidase to target-specific antibody and without provision of an exogenous source of hydrogen peroxide (xanthine oxidase plus xanthine) and halide (potassium iodide). The known ability of human myeloperoxidase to bind to C. albicans (11) may replace the need for specific antibody to direct this peroxidase to this fungal target.

The mechanism of the therapeutic influence of myeloperoxidase in the murine model of renal candidiasis was not directly determined. Information available on this point is limited to our observation that the therapeutic effect of myeloperoxidase could be abrogated by complexing the enzyme with soluble yeast cell wall mannan. However, based upon this observation and our knowledge of the importance of binding of myeloperoxidase to C. albicans for effective candidacidal activity of the enzyme in vitro (11), we suggest that the free enzyme may have had a direct cytotoxic effect against the pathogen in vivo.

We have reported that myeloperoxidase-mediated killing of C. albicans is grossly augmented in vitro when the enzyme is bound to the yeast (11) and that binding of myeloperoxidase to the organism involves in part an ionic interaction of the cationic enzyme with anionic cell wall mannan (12). We have also reported that the candidacidal effect of myeloperoxidase is inhibited when the enzyme is complexed with soluble mannan (11), an interaction which does not affect the catalytic activity of the enzyme (13), and that the myeloperoxidase-mannan complex may bind to leukocytes. Myeloperoxidase circulating as an enzyme-mannan complex in vivo would therefore be predicted to be unable or unavailable to bind to the target pathogen and to be ineffective as a direct fungicidal agent. We would also propose that mannan present in tissue fluids of patients with candidiasis might interfere with candidacidal effects of endogenous extracellular myeloperoxidase by the same mechanism.

The results described in this report demonstrate a significant therapeutic effect of exogenous human myeloperoxidase in a murine model of chronic renal candidiasis and suggest that exogenous myeloperoxidase may also be effective in treatment of at least certain forms of candidiasis in humans. Our results also demonstrate an important role for the mannan-binding function of myeloperoxidase for effective treatment of candidiasis in this model and suggest a mechanism of inhibition of the candidacidal effect of free enzyme in vivo by mannan accumulating in tissue fluids.

Initial experiments were conducted to determine a dose of C. albicans blastospores which would produce chronic renal candidiasis and a slow rate of mortality of the injected mice. Injection of 10⁶ organisms per animal produced 100% mortality by day 10 postinfection. Injection of 10⁷ organisms per animal produced 75% mortality over the subsequent 60-day observation period. Injection of 10⁸ organisms per animal produced only 10% mortality over this period. Observations made on autopsy of animals that died after receiving 10⁶ organisms suggested that they had expired as a consequence of renal candidiasis. On the average, 2 × 10⁶ organisms were recovered per mg of renal tissue, as determined by pour plate methodology. In contrast, no viable fungi were recovered from liver, lung, and spleen tissues. Based upon these results we chose to use an inoculation dose of 10⁶ blastospores per mouse for experiments to test the therapeutic influence of exogenous myeloperoxidase.

The time course of survival of mice infected with C. albicans as described is illustrated in Fig. 1. Forty percent of the mice survived to 20 days postinfection; survival was reduced to 25% over the subsequent 40 days of observation. Intraperitoneal injection of 10 mU of myeloperoxidase 1 day after injection of the C. albicans organisms was observed to increase survival of the infected mice to 80% over the full 60-day observation period. Intraperitoneal injection of 20 mg of soluble cell wall mannan had no influence on survival of the infected mice (data not shown). Administration of 10 mU of myeloperoxidase together with 20 mg of cell wall mannan, however, totally abrogated the therapeutic effect of the enzyme. These results are summarized in Table 1.

**DISCUSSION**

The results described in this report demonstrate a significant therapeutic effect of exogenous human myeloperoxidase in a murine model of chronic renal candidiasis and suggest that exogenous myeloperoxidase may also be effective in treatment of at least certain forms of candidiasis in humans.

**RESULTS**

**FIG. 1.** Time course of survival of mice receiving 10⁶ C. albicans blastospores by tail vein injection followed 1 day later by an intraperitoneal injection of phosphate-buffered saline (○), 10 mU of human myeloperoxidase (□), or 10 mU of myeloperoxidase plus 20 mg of soluble C. albicans cell wall mannan (■). Twenty 6- to 8-week-old male Swiss-Webster mice were used for each treatment group.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Survival</th>
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<tr>
<td>C. albicans plus saline</td>
<td>25 (5/20)</td>
</tr>
<tr>
<td>C. albicans plus myeloperoxidase</td>
<td>80 (16/20)</td>
</tr>
<tr>
<td>C. albicans plus myeloperoxidase plus mannan</td>
<td>35 (7/20)</td>
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* Survival of the mice was followed over a 60-day period after injection of the pathogen. The numbers within parentheses denote the number of animals surviving relative to the total number of animals in each treatment group.

* A total of 10⁶ C. albicans 2252 (ATC 44806) blastospores in a volume of 0.5 ml of phosphate-buffered saline was injected into the tail vein of each mouse.

* One day after injection of the blastospores, 0.5 ml of phosphate-buffered saline was injected intraperitoneally.

* One day after injection of the blastospores, 0.5 ml of phosphate-buffered saline containing 10 mU of purified human myeloperoxidase was injected intraperitoneally.

* One day after injection of the blastospores, 0.5 ml of phosphate-buffered saline containing 10 mU of purified human myeloperoxidase and 20 mg of soluble C. albicans 2252 cell wall mannan was injected intraperitoneally.
Given the current lack of good antifungal drugs and the results described in this report, we suggest that further experimentation to evaluate the application of exogenous myeloperoxidase to treatment of Candida spp. and other infections is warranted.

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