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"Animal safety profile of pure and formulated porcine myeloperoxidase"

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Abstract

A cell free oxidant generating system containing porcine myeloperoxidase (MPO) has been developed as a local/topical antimicrobial. The MPO system is rapidly microbicidal against a broad range of bacteria, fungi, spores, and viruses in vitro, and demonstrates rapid decrease in bacterial challenge after application onto wounds in vivo. Excellent efficacy of the MPO system both *in vitro* and *in vivo* led us to investigate the safety profile of the pure and formulated MPO.

The objectives of these studies were to develop safety profiles for pure MPO and antimicrobial formulations of MPO. These MPO formulations were designed with necessary co-factors for optimum antimicrobial activity. Toxicology end-points including morbidity, mortality, histopathology, irritation and sensitization potential, and genotoxicity of pure and formulated MPO were evaluated in regulatory driven studies.

Solutions of the pure MPO (2.35 mg MPO/mL) were evaluated for oral, dermal and pulmonary toxicity and irritation potential to skin and eyes after a single dose to young adult male and female Crl:CD®(SD)IGS BR rats or adult male and female Crl:CD®(SD)IGS BR rabbits. Rats were observed after oral or intratracheal administration at doses of 47 or 2.4 mg/kg of pure MPO, respectively over a period of 14 days for signs of adverse effects. In addition, solutions of MPO were applied to the shaved skin of rabbits at a dose of 12 mg/kg (2 mg/cm2) or placed in the eyes (0.23 mg/eye). Animals were observed for signs of toxicity over 14 days or for signs of irritation for 72 hours. Results indicate that solutions of MPO are non-toxic and non-irritating to eyes when administered at the maximum feasible dose. The MPO solution produced only a very slight erythema reaction after dermal application in one out of 3 rabbits which cleared within 24 hours.

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The potential for immune mediated reactions was evaluated in a dermal sensitization model in which young adult male Crl:(HA)BR guinea pigs were given intradermal and dermal applications of MPO solutions. The MPO solution did not produce an immune-mediated hypersensitivity, and thus MPO is not considered a skin sensitizer. The potential for MPO solutions to induce mutations was evaluated both in vivo using a murine bone marrow micronucleus assay and in vitro using a mouse lymphoma forward mutation assay. Neither study produced mutations above background demonstrating that MPO is not genotoxic.

The potential toxicity of the MPO formulated system was also evaluated. In these studies young adult Sprague-Dawley Crl:CD® (SD) (IGS)BR male and female rats were given 1 mg MPO/mL formulations at the maximum feasible dose by intratracheal instillation, dermal application to abraded skin, or subcutaneous application under the margin of an incisional wound. A comprehensive toxicological evaluation including morbidity, mortality, sensitization and irritation potential, abnormal clinical signs, clinical pathology (clinical chemistry and hematology) and microscopic examination of over 40 tissues was made. In rats given formulations by intratracheal instillation (1 mg/kg) or dermal application (2.5 mg/kg) comprehensive evaluations were conducted at 24 hours and 14 days after dosing to assess both early and delayed effects. No adverse effects were noted for any of the clinical pathology parameters evaluated or in any of the tissue sections examined microscopically at either time-point.

The potential for an immune-mediated sensitization to the MPO formulation was evaluated using the local lymph node assay. The formulation was applied to the ears of CBA/J female mice, (6-7 weeks old) once per day for three days in order to stimulate the proliferation of cells in the lymph nodes. The MPO formulation did not produce a proliferation of lymph node tissue and thus can be considered non-sensitizing using this assay.

In summary, safety studies conducted to date, in multiple animal species and different routes of administration, demonstrate that MPO and MPO formulations appear non-toxic, non-irritating, non-genotoxic, and non-sensitizing. The unique rapid microbicidal activity of the MPO system suggests a high margin of safety to support a wide variety of therapeutic applications.

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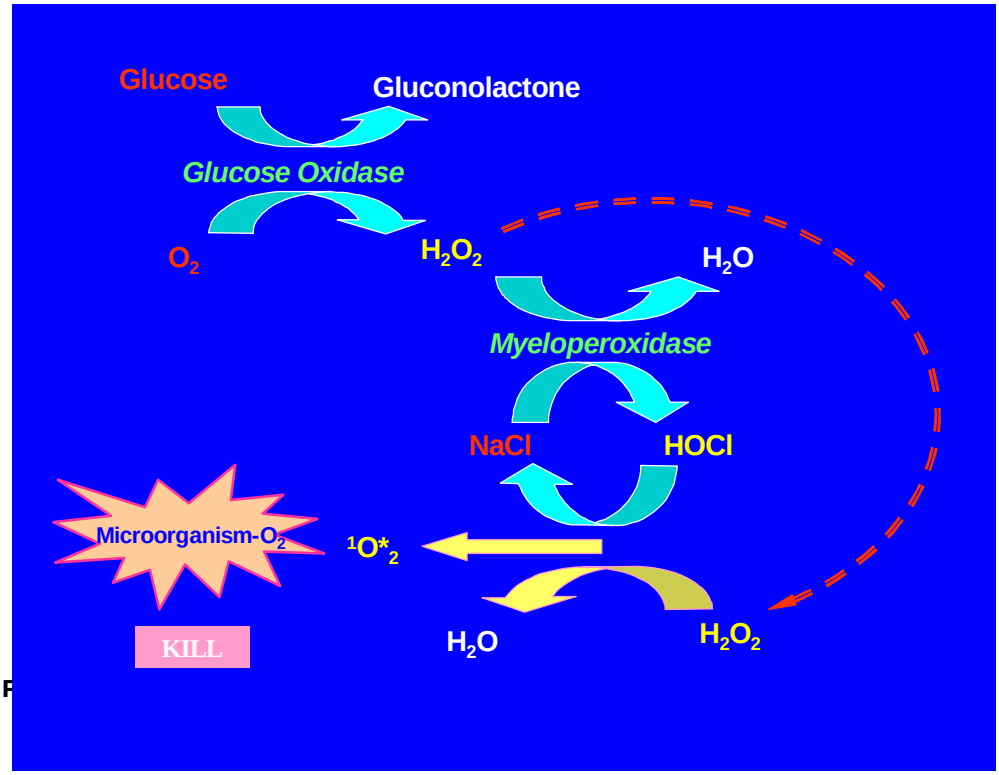
Introduction

- Myeloperoxidase (MPO), a neutrophilic protein, is stored in azurophilic granules and released into the phagosome during phagocytosis.
- MPO uses superoxide anion and hydrogen peroxide to form reactive oxidative species that cause cell damage.
- A cell free, oxidant generating, enzyme system containing MPO has been developed which exploits this natural antimicrobial system (ExOxEmis, Inc., Little Rock, AR).
    - The mechanism of action (Figure 1) involves the use of hydrogen peroxide, generated to convert chloride into hypochlorous acid.
    - Hypochlorous acid either participates in the direct halogenation of target cell components, or reacts with a second hydrogen peroxide molecule to yield singlet oxygen.
    - Singlet oxygen, a broad spectrum oxygenating electrophile, is a potent bactericidal agent [1,2].



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Objectives

The objective of the present study was to investigate safety of the MPO enzyme and the MPO formulation in animals after several routes of administration.

Methods

Strategy for Safety Studies

- Conduct single-dose studies with purified MPO to determine the safety profile of the pure protein in order to evaluate:
  - Morbidity and mortailty
  - Irritation potential
  - Immunogenicity
  - Mutagenicity

- Conduct single dose safety studies on MPO formulations:
  - Detailed toxicity studies after relevant routes of exposure in rats and mice
  - Immunogenic potential of complete formulation

- All studies were conducted using Good Laboratory Practice (GLP) Guidelines and standard Food and Drug Administration (FDA) approved protocols.

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Results

Summary of Results for Safety Studies with MPO

Study	Test System	Dose	Findings
Acute Oral Toxicity	Young adult Male and Female Crl:CD®(SD)IGS BR Rats	47 mg MPO/kg 2.352 mg MPO /mL 20 mL/kg	No mortality
Acute Dermal Toxicity	Adult Male and Female Hra:(NZW)SPF Rabbits	12 mg MPO/kg for 24 hr 2.352 mg MPO/mL 5.0 mL/kg 0.07 mL/cm^2 or 0.1646 mg MPO/ cm^2	No mortality; Slight dermal irritation in 6/10 rabbits
Acute Pulmonary Toxicity	Young adult Male and Female Crl:CD®(SD)IGS BR Rats	2.352 mg MPO/kg 2.352 mg MPO/mL 1 mL/kg	No mortality
Dermal Irritation	Adult Female Hra:(NZW)SPF Rabbits	0.5 mL over 6.25 cm^2 for 4 hr; 2.352 mg MPO/mL 0.1882 mg MPO/cm^2	Slight dermal irritation in 1/10 rabbits; Primary dermal irritation index 0.1 (slightly irritating)
Ocular Irritation	Adult Female Hra:(NZW)SPF Rabbits	0.1 ml/right eye for 4 hr 2.352 mg MPO/mL; 0.2352 mg MPO/right eye	No positive irritation reactions
Dermal Hypersensitivity	Adult Male Crl:(HA)BR Guinea Pigs	2.352 mg/mL 0.1 ml intradermal injection or topical application	Not sensitizing
Clastogenicity	Male and Female Crl:CD-1®(ICR)BR mice	5.4, 10.9, and 21.7 mg MPO/kg 10 mL/kg	Not cytotoxic No increased micronuclei
Mutagenicity	Mouse lymphoma L5178Y cell line	21.2 ng MPO/mL to 0.0109 mg MPO/mL (for cytotoxicity) 9.98 ng MPO/mL to 0.0217 mg MPO/mL for genotoxicity	No cytotoxicity No increased mutant frequency

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Results

Summary of Results for Safety Studies with MPO Formulation

Study	Test Animal	Dose	Findings
Expanded Acute Pulmonary Toxicity	Young Adult Male and Female Crl:CD® (SD) (IGS)BR Rats	0.25 or 1 mg MPO/kg 1 mL/kg 0.25 or 1 mg MPO/mL	No mortality; no adverse clinical signs; no abnormal gross, clinical pathology or microscopic findings
Expanded Acute Dermal Toxicity on Abraded Skin	Young Adult Male and Female Crl:CD® (SD) (IGS)BR Rats	2.5 mg MPO/kg for 24 hr 2.5 mL/kg 1 mg MPO/mL	No mortality; no adverse clinical signs; no abnormal gross, clinical pathology or microscopic findings
Expanded Acute Dermal Toxicity on Incised Skin	Young Adult Male and Female Crl:CD® (SD) (IGS)BR Rats	2.5 mg MPO/kg 2.5 mL/kg 1 mg MPO/mL	No mortality; no adverse clinical signs; no abnormal gross, clinical pathology or microscopic findings
Local Lymph Node Assay	Young Adult Female CBA/J Mice	Three repeated applications of 0.025 mL to ears 0, 0.2, 0.3, 0.4, 0.5 mg MPO/mL 1:1 formulation:propylene glycol	No sensitization

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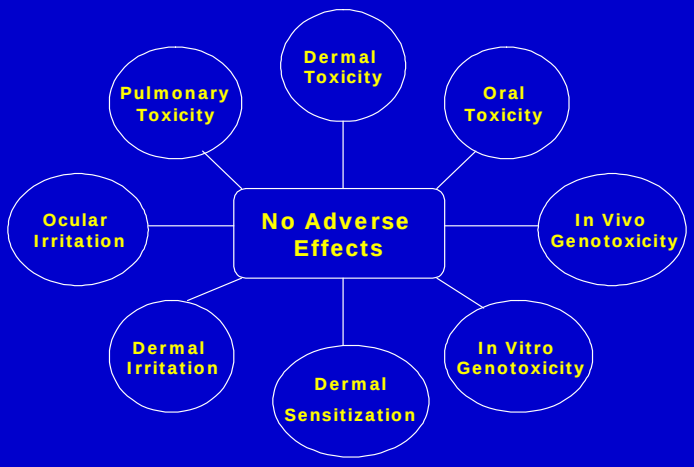
Conclusions

Safety Studies with MPO in Multiple Animal Species Show No Adverse Effects At Maximal Feasible Dose



Safety Demonstrated After Multiple Routes of Administration for the MPO Formulation System

Route of Exposure	Regulatory Implication *
Applied to abraded skin	NOAEL 2.5 mg MPO/kg
Applied under skin incision	NOAEL 2.5 mg MPO/kg
Intratracheal instillation	NOAEL 1 mg MPO/kg
Applied to ears	Not a skin sensitizer

*NOAEL = No observed adverse effect level

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References

(1) Chika, K., Makiuchi, M., Miyazaki, J., Fujinaga, T., and Kakinuma, K. 1999. Physiological production of singlet molecular oxygen in the myeloperoxidase-H202-chloride system. FEBS Letters 443, 154-158.

(2) Tatsuzawa, H., Maruyama, T., Hori, K., Sano, Y., and Nakano, M. 1999. Singlet oxygen as the principal oxidant in myeloperoxidase-mediated bacterial killing in neutrophil phagosome. Biochem. Biophys. Res. Commun. 262, 467-650.

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