Inflammation, DNA-centered radicals, and oxidative genotoxicity: The role of HOCl produced by myeloperoxidase in carcinogenesis

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Introduction

Myeloid cells (macrophages and neutrophils) infiltrate and synthesize myeloperoxidase (MPO) at sites of inflammation, which might produce gentotoxicity at surrounding tissues.

Previously, we found that "*freezing*" LPS-triggered macrophage activation with the nitrone spin trap 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO), prevented cell activation and death. Oxidation of proteins and genomic DNA was also blocked, with formation of protein- and DNA-DMPO nitrone adducts, as assessed by DMPO-based immuno-spin trapping with the polyclonal anti-DMPO serum. Interestingly, confocal microscopy analysis of these cells showed that MPO, genomic DNA, and DNA-DMPO nitrone adducts co-localized in the nuclear protuberances formed in LPS-activated macrophages.

Based on these observations, and the fact that DNA is negatively charged and MPO is a cationic protein, we hypothesized that uptaken or newly synthesized MPO induces oxidative mutagenesis when activated at sites of inflammation. We also tested whether resveratrol can prevent MPO-driven DNA-centered radicals and further mutagenesis.

Methods and Results

In order to understand MPO-induced formation of DNA-centered radicals, we studied DNA-DMPO nitrone adducts in calf thymus DNA treated with micromolar concentrations of hypochlorous acid (HOCl) added as a bolus or generated *in situ* by the MPO/H_2O_2/Cl^- system in the presence of DMPO.

We also investigated DNA-DMPO nitrone adducts inside living cells containing MPO, such as in HL-60 cells exposed to glucose/glucose oxidase (GO) or in RAW264.7 cells activated with LPS and then exposed to a phorbol ester (PMA).

In addition, we used A549 human airway epithelial cells pre-loaded with human MPO. When these cells were exposed to glucose/GO, the frequency of 6-thioguanine-resistant cells—with the hypoxanthine-guanine phosphoribosyl transferase (*hrpt*) gene mutation, increased.

The formation of DNA-centered radicals and HRPT mutation frequency in our cell systems decreased by each of the following: the NADPH oxidase inhibitor apocynin; the MPO inhibitors salicylhydroxamic acid (SHA) and 4-aminobenzoic acid hydrazide (ABAH); the cell-permeable HOCl scavenger resveratrol; and DMPO, which traps DNA-centered radicals and prevents further oxidation.

Possible sites of intervention to stop and study myeloperoxidase-driven damage to the genome at sites of inflammation

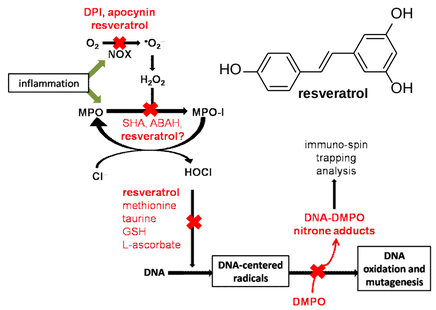

Figure 1: Rationale for the study of MPO-driven gentoxic damage and interventions: In the site of inflammation superoxide radical anion is produced by NADPH oxidase 2 (NOX2) in inflammatory cells and then dismutated to H_2O_2. MPO uses H_2O_2 to oxidize chloride to HOCl (chlorination cycle of MPO). HOCl or chloramines formed in the medium can diffuse inside tissue cells, or be formed inside cells that take up MPO, to produce DNA-centered radicals. In red, possible sites of interventions and study of DNA-centered radicals are shown. Insert shows the chemical structure of resveratrol.

HOCl induces DNA-centered radicals

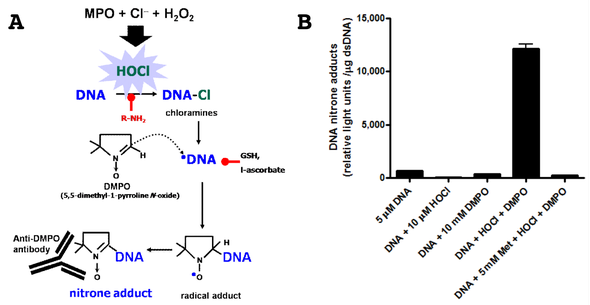

Figure 2: HOCl oxidizes DNA to form DNA-centered radicals as intermediates. A) DMPO-based immuno-spin trapping analysis of DNA-centered radicals produced during the MPO-driven, HOCl-mediated oxidation of DNA; **B**) ELISA analysis of DNA-centered radicals induced by HOCl using the anti-DMPO antibody.

Kinetic study of DNA-centered radicals induced by HOCl using immuno-spin trapping

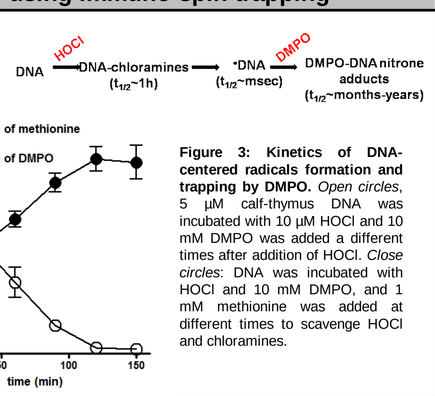

Figure 3: Kinetics of DNA-centered radicals formation and trapping by DMPO. *Open circles*, 5 µM calf-thymus DNA was incubated with 10 µM HOCl and 10 mM DMPO was added a different times after addition of HOCl. *Close circles*: DNA was incubated with HOCl and 10 mM DMPO, and 1 mM methionine was added at different times to scavenge HOCl and chloramines.

DNA-centered radicals induced by MPO chlorination activity is blocked by resveratrol

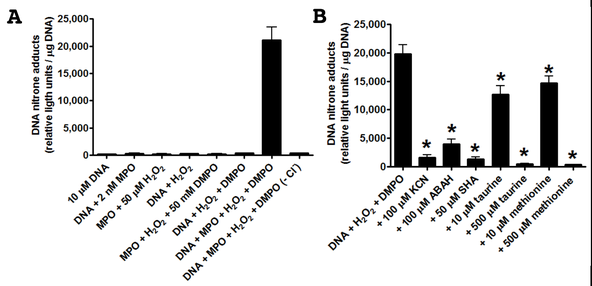

Figure 4: MPO-driven, HOCl-induced DNA-DMPO nitrone adducts. A) Calf thymus DNA was incubated with MPO and H_2O_2 followed by addition of 50 mM DMPO. Reaction was performed in 10 mM sodium phosphate buffer, pH 7.4 with or without 100 mM Cl^-. **B**) same as A, but inhibitors of MPO and scavengers of HOCl were added at the concentrations indicated in the figure. Addition of reagents was performed before H_2O_2 addition. Nitrone adducts were determined by ELISA. Asterisks indicate P<0.05 with respect to no inhibitor or scavenger added.

Resveratrol inhibits DNA-centered radicals induced by the chlorination cycle of MPO by scavenging HOCl

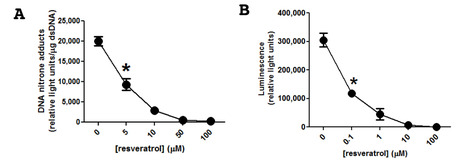

Figure 5: Resveratrol inhibits DNA oxidation by scavenging HOCl. A) Resveratrol inhibits HOCl-induced DNA-DMPO nitrone adducts. **B**) Resveratrol inhibits HOCl-induced luminol chemiluminescence (5 µM luminol/5 µM HOCl, 2 mins reaction).

Genomic DNA-centered radicals in HL-60 cells are produced by the chlorination cycle of MPO

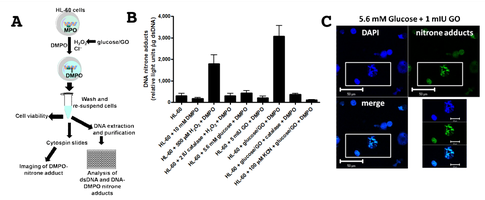

Figure 6: MPO produces DNA-centered radicals in HL-60 cells. **A**) shows the schematic procedure to study DNA-centered radicals induced by incubation of HL-60 cells to H_2O_2. **B**) ELISA analysis in total genomic DNA isolated from HL-60 cells treated as in A. **C**) confocal microscopy analysis of HL-60 cells treated with H_2O_2 showing the unique nuclear localization of DNA-DMPO nitrone adducts. Inserts, show a single plane image of a typical cell.

Epithelial cells uptake MPO at sites of inflammation what makes them vulnerable to H_2O_2 in the site of inflammation: intervention with resveratrol

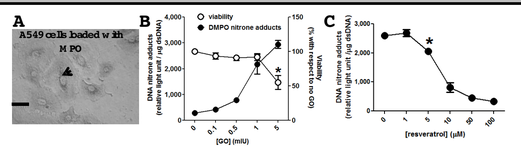

Figure 7: Uptake of MPO by epithelial cells can produce DNA-centered radicals at sites of inflammation. A) A549 human lung epithelial cells were loaded with 10 nM human MPO for 24 h and then taken up MPO was imaged using an antibody anti-MPO. **B**) as A but cells were incubated with 5.6 mM glucose/glucose oxidase (GO) and DMPO for 1 h followed by measurement of viability (trypan blue exclusion) and DNA-nitrone adducts by ELISA in DNA extracted from the cells after treatment. **C**) same as B, but resveratrol was added 30 min before 5 mIU of GO.

LPS-elicitation of macrophages induces MPO that makes them vulnerable to genomic damage upon activation

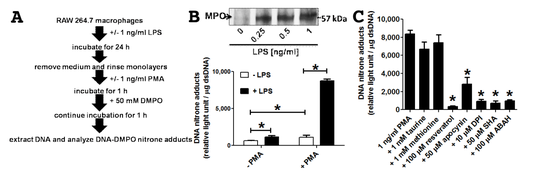

Figure 8. Resveratrol protects inflammatory cells against MPO-driven genotoxicity. A) RAW 264.7 cells were treated as shown in the scheme. **B**) upper panel, Western blot showing the induction of MPO expression in macrophages incubated with LPS according to scheme in A. Lower panel shows the ELISA analysis of DNA-DMPO nitrone adducts in the DNA extracted from LPS-treated macrophages incubated with the phorbol ester PMA as shown in A. **C**) Shows the effect of HOCl scavengers, NOX2 inhibitors and MPO inhibitors on PMA-induced DNA-DMPO nitrone adducts in macrophages elicited with LPS.

DMPO inhibits mutagenesis induced by HOCl by trapping DNA-centered radicals

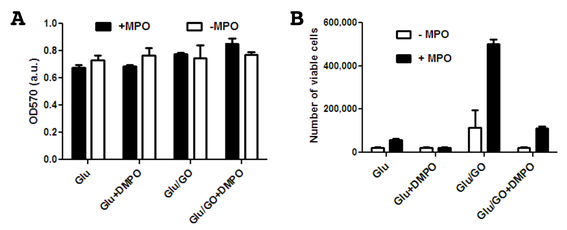

Figure 9. DMPO inhibits MPO-driven, HOCl-triggered mutation in A549 human lung epithelial cells. A) A549 cells were loaded with MPO followed by incubation with 5.6 mM glucose/ 1 mIU/ml glucose oxidase (GO) and/or 25 mM DMPO as indicated in the figure. After 1 h incubation, the viability of the cells was determined using the MTT assay. **B**) Other T75 flasks were treated as in A and then rinsed with PBS and incubated in fresh medium (F12K with 5% FCS) for 7 days. After that, cells were incubated with fresh medium containing 5 µg/ml 6-thioguanine (6-TG) for 14 days to select cells having the mutation in the **hypoxanthine-guanine phosphoribosyl transferase (*hrpt*) gene**. Mutant cells are resistant to 6-TG and proliferate forming colonies. Cell were trypsinized and counted in an hemocytometer.

Conclusions

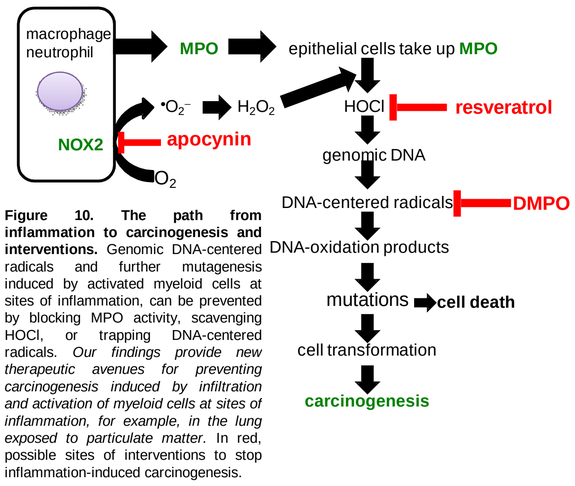

Figure 10. The path from inflammation to carcinogenesis and interventions. Genomic DNA-centered radicals and further mutagenesis induced by activated myeloid cells at sites of inflammation, can be prevented by blocking MPO activity, scavenging HOCl, or trapping DNA-centered radicals. *Our findings provide new therapeutic avenues for preventing carcinogenesis induced by infiltration and activation of myeloid cells at sites of inflammation, for example, in the lung exposed to particulate matter*. In red, possible sites of interventions to stop inflammation-induced carcinogenesis.

Acknowledgement

This work was supported in part by the National Institute of Environmental Health Sciences (5R00ES015415-03) and OMRF/PHF starting up fund to DC Ramirez.

Presbyterian Health Foundation

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