Dimethylsulfoxide (DMSO) induces downregulation of heme oxygenase-1 (HO-1) in HL-60 cells: involvement of HO-1 in HL-60 cell differentiation

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Heme oxygenase-1 (HO-1), an inducible enzyme with broad tissue expression, is well-regulated in response to hematopoietic stress and preserves vascular homeostasis. We investigated the involvement of HO-1 in HL-60 cell differentiation. Dimethyl sulfoxide (DMSO) completely decreased HO-1 expression in a time-dependent manner, but clearly induced HL-60 cell differentiation, as evidenced by a marked increase in CD11b expression. Interestingly, zinc protoporphyrin (ZnPP), a strong inhibitor of HO-1, induced HL-60 cell differentiation. In contrast, treatment with cobalt protoporphyrin (CoPP), an activator of HO-1, decreased CD11b expression. Additionally, ZnPP downregulated HO-1 protein expression in HL-60 cells, whereas CoPP induced upregulation. These results suggest that HO-1 might have a negative function in DMSO-induced HL-60 cell differentiation. This study provides the first evidence that HO-1 plays an important role in DMSO-induced HL-60 cell differentiation. [BMB reports 2011; 44(11): 753-757]

INTRODUCTION

Heme oxygenase-1 (HO-1), an inducible rate-limiting enzyme in heme catabolism, is regulated in response to hematopoietic stress and preserves vascular homeostasis. HO-1 metabolizes the heme porphyrin ring to yield equimolar amounts of biliverdin, free iron (Fe2+), and carbon monoxide (CO). In mammals, biliverdin is rapidly converted by biliverdin reductase into bilirubin (1-3). Consequently, HO-1 plays a critical role in regulating the levels of cellular hemoproteins, including nitric oxide synthase, soluble guanylate cyclase, cytochrome P450, and cyclooxygenase, not only for the intracellular heme pool available for apo-hemoproteins (4), but possibly also at the CO level, a downstream HO-1 effector molecule (5). HO-1 deficiency is associated with tissue pathology in patients with atherosclerosis and other inflammatory conditions linked with intravascular thrombosis such as septic shock, hypoxia, and graft rejection. During hematopoietic stress, hematopoietic stem cells (HSCs) are recruited into the cell cycle to differentiate and produce mature blood cells to meet the immediate challenge (6, 7). HO-1 is a critical regulator of the stress response in HSCs by controlling the level of its substrate (heme) and bioactive products (biliverdin/bilirubin and CO), particularly under stress conditions (8). These findings indicate that HO-1 plays an essential role in hematopoiesis under stress conditions.

Dimethyl sulfoxide (DMSO) is a simple amphipathic molecule with a polar domain and two non-polar groups (9). It is widely used as a drug therapy vehicle for various diseases, including gastrointestinal disorders (10), dermatological disorders (11-13), pulmonary adenocarcinoma (14), musculoskeletal disorders (15), traumatic brain edema (16), schizophrenia (17), interstitial cystitis (18), chronic prostatitis (19), rheumatologic disorders (20, 21), and amyloidosis (22-27). In particular, DMSO has been used to treat cancer for several decades. HL-60 cells, a human promyelocytic leukemia cell line, differentiate into granulocyte-like or monocyte/macrophage-like cells and lose their proliferative properties when treated with DMSO (28). Furthermore, DMSO arrests the cell cycle of several human lymphoid cell lines at the G1 phase (29). Accordingly, it seems likely that DMSO could be used as a differentiation therapy supplement for leukemia.

Metalloporphyrins are a class of compounds in which the central iron of heme is replaced by various metals such as zinc, cobalt, chromium, manganese, or tin (30). These metalloporphyrins work as HO-1 reaction competitive inhibitors because of their inefficient binding to molecular oxygen, which prevents
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Fig. 1. DMSO downregulates heme oxygenase-1 (HO-1) in HL-60 cells but increases CD11b expression. HL-60 cells (5 × 10⁵) were stimulated with 1.3% DMSO for the indicated times. (A), HO-1 protein levels were analyzed by Western blotting using β-actin as the loading control. Electrophoretic bands were analyzed with an LAS-1000 (Fujifilm, Tokyo, Japan). The right density graph indicates the ratio of HO-1/β-actin in the Western data (B), CD11b expression was measured using flow cytometry.

HO-1 from degrading the metalloporphyrins (30). Zinc protoporphyrin (ZnPP) and tin protoporphyrin (SnPP) act as HO-1 inhibitors. In contrast, cobalt protoporphyrin (CoPP) induces and activates HO-1, whereas copper protoporphyrin (CuPP) does not inhibit HO-1 activity (31–33). The different activities of these metalloporphyrins make them useful tools for elucidating the potential role of HO-1 in DMSO-induced differentiation of acute promyelocytic leukemia cells. Understanding the mechanisms that control cell differentiation is essential for understanding and controlling diseases such as acute myeloid leukemia. Inducing leukemic cell differentiation in mature cells represents a major strategy for treating leukemia.

We performed in vitro experiments using the HL-60 cell line to investigate the involvement of HO-1 in leukemia cell differentiation. Our data indicated that HO-1 plays an important role in HL-60 cell differentiation.

RESULTS AND DISCUSSION

DMSO downregulates HO-1 in HL-60 cells but increases CD11b expression

DMSO induces differentiation of the human monoblast leukemia cell line U937 (34), a human ovarian adenocarcinoma cell line (35), and in human promyelocytic leukemia HL-60 cells (36). Furthermore, a recent study showed a dose-dependent suppression of HO-1 protein production by DMSO using immunoblotting in the U937 human myelomonocytic lymphoma cell line (37), indicating that HO-1 is involved in HL-60 cell differentiation.

Accordingly, we first examined whether HO-1 protein expression is also downregulated by DMSO in a human leukemia cell line. HL-60 cells (5 × 10⁵) were treated with 1.3% DMSO for various periods of time and used to prepare whole cell extracts for HO-1 Western blotting. DMSO caused an HO-1 time-dependent decrease, with a significant decline within 24 h after treatment that persisted until 72 h post-treatment (Fig. 1A). We also measured CD11b cell surface expression, a HL-60 cell differentiation marker, to determine the degree of HL-60 cell differentiation in response to DMSO. Treatment with 1.3% DMSO increased CD11b expression in a time-dependent manner, starting after 2 days and reaching a maximum 3 days after treatment (Fig. 1B). These results suggest that HO-1 may have a negative function in DMSO-induced HL-60 cell differentiation.

ZnPP induces HO-1 downregulation in HL-60 cells, whereas CoPP induces upregulation

HO-1 protein expression and activity are both diminished by ZnPP treatment in mouse tumors (38). Fang et al. showed that although more intense ZnPP treatment increases HO-1 levels in sarcoma tumors, the treatment also significantly inhibits HO-1 activity in tumors (39). Yang et al. similarly found that ZnPP treatment increases HO-1 protein expression and decreases HO-1 activity. Because heme is a very strong inducer of HO-1, heme accumulation from the inhibition of HO-1 activity could result in increased HO-1 expression (40). ZnPP acts as an HO-1 inhibitor, whereas CoPP induces and activates HO-1 in the LL2 mouse lung cancer cell line (38). In contrast, ZnPP causes the highest HO-1 induction among ZnPP, tin (SnPP), and chromium protoporphyrins (CrPP) in the HA-1 hamster fibroblast line (40). Consequently, we examined the effect of ZnPP and CoPP on HO-1 protein expression in HL-60 cells. HL-60 cells (5 × 10⁵) were treated with 10 μM ZnPP and 10 μM CoPP for various times and used to prepare whole cell extracts for Western blotting of HO-1. ZnPP decreased HO-1 to levels lower than those of the control, and these responses were persistent for 72 h (Fig. 2). In contrast, CoPP increased HO-1 protein to levels greater than those by
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Fig. 2. Effect of portoporphyrin on heme oxygenase-1 (HO-1) expression in HL-60 cells. HL-60 cells (5 × 10⁵) were stimulated with cobalt protoporphyrin (CoPP) (10 μM) or zinc protoporphyrin (ZnPP) (10 μM) for the indicated times. HO-1 protein levels were analyzed by Western blotting. Electrophoretic bands were analyzed with an LAS-1000 (Fujifilm, Tokyo, Japan). The lower density graph indicates the ratio of HO-1/β-actin for the Western data.

Fig. 3. Effect of protoporphyrin on HL-60 cell viability. Cells were cultured in 96-well plates to 70% confluence and then incubated with the indicated concentrations of protoporphyrin for 72 h. The MTT assay was used to assess cell viability. The optical density value of the control was regarded as 100%. Data points are the mean ± SE of three independent experiments.

ZnPP, and these responses continued until 48 h post-treatment (Fig. 2). We designed the experiment to avoid cytotoxicity interference with the reagent. The effect of protoporphyrins on HL-60 cellular toxicity was assessed using the MTT assay. Treatment of HL-60 cells with protoporphyrins (0-10 μM) for 72 h resulted in no significant effect on cell viability (Fig. 3).

ZnPP increases CD11b expression in HL-60 cells, whereas CoPP decreases expression
ZnPP diminishes increased cell proliferation seen in leukemia (41), and zinc porphyrins are potent inhibitors of hematopoiesis in animal and human bone marrow (42). We investigated whether ZnPP could induce HL-60 cell differentiation. Treatment with 10 μM ZnPP dramatically boosted CD11b cell surface expression compared with that of 10 μM CoPP, which decreased cell CD11b surface expression (Fig. 4). CD11b expression in response to CoPP and ZnPP changed at 24 h after protoporphyrin treatment, and these changes persisted until 3 days after treatment.

In conclusion, DMSO completely induced downregulation of HO-1 expression in differentiated HL-60 cells. Additionally, ZnPP, an inhibitor of HO-1, also strongly increased CD11b expression. In contrast, CoPP, an activator of HO-1, decreased CD11b expression (Fig. 3). These results suggest that downregulation of HO-1 might be necessary for DMSO-induced HL-60 differentiation. If HO-1 is an important regulator of differentiation and proliferation in HL-60 cells, it represents a potential chemotherapy target. Further study is needed to elucidate the control mechanisms of HO-1 expression involved in the differentiation and proliferation of HL-60 cells. This study provides the first evidence that HO-1 plays an important role in DMSO-induced differentiation of HL-60 cells. Control of the HO-1 protein may be a useful strategy for managing human leukemia.

MATERIALS AND METHODS

Materials
CD11b monoclonal goat anti-mouse antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). DMSO, ZnPP, CoPP, Hank's balanced salt solution, and EDTA were obtained from Sigma Chemical Co. (St. Louis, MO, USA). RPMI 1640 and fetal calf serum were purchased from Gibco-BRL (Gaithersburg, MD, USA).

Cell culture
HL-60 cells were obtained from the American Type Culture Collection (Rockville, MD, USA) and cultured in RPMI 1640 containing 10% fetal calf serum, 2 mM glutamine, and antibiotics (penicillin G 60 mg/L, streptomycin 100 mg/L, and amphotericin B 50 μg/L) in a humidified atmosphere of 5% CO₂ and 95% air.

MTT assay for cell viability
The effect of cordycepin on cell viability was determined using the MTT assay. Viability of cultured cells was determined by reduction of MTT to formazan. Briefly, 5 × 10⁵ cells/well were incubated in a 96-well plate and treated with various concen-
tations of ZnPP or CoPP. After a 72 h incubation, the cells were washed twice with PBS. MTT (0.5 mg/ml PBS) was added to each well, and incubated at 37°C for 30 min. DMSO (100 μl/well) was added to dissolve the formazan crystals. The plate was read in a microplate reader (Model 3550, BIO-RAD, Richmond, CA, USA) at 570 nm.

**Western blot analysis for HO-1 protein expression**

HL-60 cells (5 × 10⁷) were treated with 1.3% DMSO, 10 μM ZnPP, and 10 μM CoPP for various times and used to prepare whole cell extracts for HO-1 and cyclin D1 Western blotting. Total protein (25 μg in each sample) in tissue homogenates was separated by electrophoresis on 12% SDS-polyacrylamide gels and transferred to Immobilon polyvinylidene difluoride membranes (Millipore Co., Ltd., Bedford, MA, USA), followed by incubation with an HO-1 polyclonal antibody (OSA-150; Stressgen, Victoria, British Columbia, Canada). The protein band that reacted immunologically with the antibody was visualized using an enhanced chemiluminescence detection (Amersham).

**FACS analysis for CD11b expression to induce differentiation**

Cell surface expression of antigen CD11b was determined by fluorescence-activated cell sorting analysis. HL-60 cells were harvested and washed once with phosphate buffered saline containing 0.5% bovine serum albumin. Aliquots of cell suspension were stained with mouse monoclonal antibody for CD11b and FITC-labeled goat anti-mouse IgG and analyzed with a Becton Dickinson flow cytometer (Franklin Lakes, NJ, USA).

**Statistical analysis**

All experimental data are shown as means ± standard deviations. The statistical analysis was performed using Student’s t-test, and P < 0.005 was considered significant.

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