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Hemin activation of innate cellular response blocks human immunodeficiency virus type-1-induced osteoclastogenesis

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ABSTRACT

The normal skeletal developmental and homeostatic process termed osteoclastogenesis is exacerbated in numerous pathological conditions and causes excess bone loss. In cancer and HIV-1-infected patients, this disruption of homeostasis results in osteopenia and eventual osteoporesis. Counteracting the factors responsible for these metabolic disorders remains a challenge for preventing or minimizing this co-morbidity associated with these diseases. In this report, we demonstrate that a hemin-induced host protection mechanism not only suppresses HIV-1 associated osteoclastogenesis, but it also exhibits anti-osteoclastogenic activity for non-infected cells. Since the mode of action of hemin is both physiological and pharmacological through induction of heme oxygenase-1 (HO-1), an endogenous host protective response to an FDA-licensed therapeutic used to treat another disease, our study suggests an approach to developing novel, safe and effective therapeutic strategies for treating bone disorders, because hemin administration in humans has previously met required FDA safety standards.

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1. Introduction

Osteoclasts are the normal bone resorbing cells. These cells release calcium and growth factors from bone to maintain normal bodily functions and contribute to physiological bone remodeling, as well as pathological bone destruction in osteoporosis and rheumatoid arthritis; thus, they represent a pharmacological target for drug development [1–5]. Extensive evidence points convincingly towards increased activity of osteoclasts and impaired activity of osteoblasts in cancer and HIV-infected patients, leading to a significant increase in the prevalence of osteoporosis [6–16].

HIV-infected patients show bone loss and osteopenia/osteoporosis during the course of the disease [17–22]. The mechanisms underlying this degenerative process are largely unclear, and it has yet to be determined how bone dysfunction is linked to HIV-1-mediated direct and/or indirect effects on osteoblasts/osteoclasts and their cross-talk regulation. In addition, development of osteopenia and augmentation of osteoporosis are reported to be associated with antiretroviral treatment [23–30], although the mechanisms involved have also not yet been elucidated. Since osteoclasts, the large multinucleated cells responsible for resorption of bone, are the mediators of continuous bone loss, identification of host factors promoting or inhibiting osteoclastic activity will facilitate designing effective therapeutic strategies against osteoporosis induced by HIV and cancer.

In this report, we demonstrate that HIV-infected monocyte-derived macrophages (MDM) are morphologically and functionally related to osteoclasts, and they exhibit increased susceptibility to multinucleated giant cell formation by receptor activator of nuclear factor kappa-B ligand (RANKL), a pivotal factor for differentiation of pre-osteoclasts into osteoclasts. In addition, we show that pharmacologically relevant concentrations of hemin inhibit HIV-induced osteoclast formation, as well as blocking RANKL function to prevent osteoclastogenesis. This is the first report to our knowledge demonstrating that hemin can serve as a potentially novel biologic in mitigating the effects of both HIV and RANKL on osteoclastogenesis, thus suggesting new strategies for developing potentially safe therapeutic interventions for the treatment of osteopenia/osteoporosis associated with HIV or other medical conditions.

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2. Materials and methods

2.1. Reagents

The FDA-approved drug, Panhematin®, was purchased from Lundbeck (manufactured by APP Pharmaceuticals) and used to induce the cytoprotective enzyme heme oxygenase-1 (HO-1). Mouse-anti-human HO-1 antibody was purchased from Enzo Life Sciences (Farmingdale, NY). The HIV-1_{Ba-L} strain was purchased from Advanced Biotechnologies, Inc., Columbia, MD.

2.2. Isolation, culture, and infection of cultured monocytes

Human monocytes were isolated from peripheral blood mononuclear cells of donors seronegative for HIV-1 and hepatitis B after leukopheresis and were purified by countercurrent centrifugal elutriation [31]. Cell suspensions contained >95% monocytes based on cell morphology in Wright-stained cytosmears, granular peroxidase, and nonspecific esterase. The cells were cultured for 5 days in DMEM supplemented with 10% FBS, 20 μg/ml gentamicin, 1000 U/ml M-CSF, and then were infected with HIV-1_{Ba-L} as previously described [32]. Cell-free culture supernatants were assayed for HIV-1-p24 using an NEN/DuPont ELISA analysis kit (PerkinElmer) according to the manufacturer's instructions.

2.3. Detection of HO-1 expression by Western blot (WB) analysis

Total protein extracts were prepared from MDM in a proprietary formulation of lysis buffer containing SDS and protease and phosphatase inhibitors (Kendrick Labs, Inc., Madison, WI). Protein concentrations were determined using BCA protein assay kits (Pierce). After addition of loading buffer, equal protein amounts (2.5 μg) of each lysate preparation were subjected to electrophoresis using 10–20% polyacrylamide gradient gels (Invitrogen Life Technologies). Proteins were transferred to nitrocellulose by electroblotting, and nonspecific sites were blocked with 5% nonfat milk in PBS, pH 7.4, containing 0.1% Tween 20 (PBST) for 18 h at 4 °C. After washing three times with PBST, the blots were incubated for 1 h at room temperature with a cocktail of mouse or rabbit-anti-human HO-1 antibody and rabbit-anti-human actin antibody, and washed three times with PBST. Transferred proteins were incubated with the ECL Western blotting detection system (GE Healthcare, Piscataway, NJ) according to the manufacturer's instructions, followed by visualization with x-ray film.

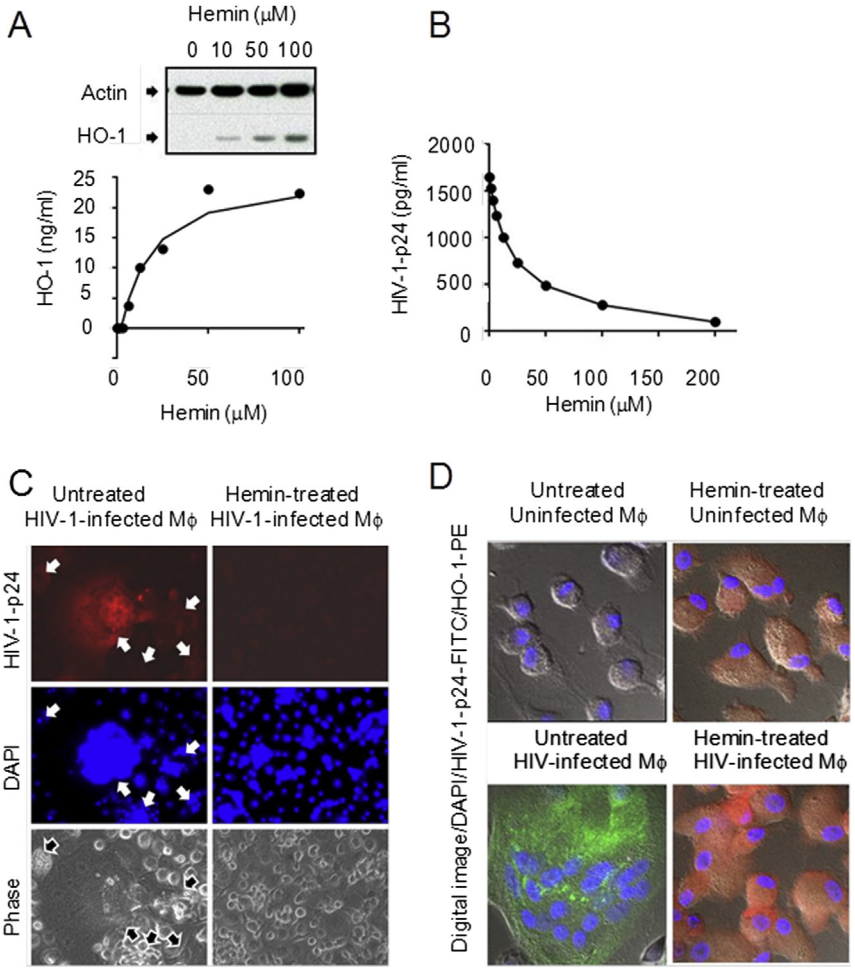

Fig. 1. HO-1 induction is inversely related to HIV infection of MDM. A. MDM treated with various concentrations of hemin and examined for cellular HO-1 induction by WB and ELISA. B. HIV-1-p24 levels in culture fluids from MDM infected with HIV in the absence or presence of various concentrations of hemin. C. HIV-1-p24 expression and nuclear staining by immunofluorescence microscopy and phase-contrast images of HIV-infected MDM cultured for 10 days in the absence or presence of 100 μM hemin. D. Confocal microscopy of uninfected and HIV-1-infected MDM cultured in the absence or presence of 100 μM hemin followed by simultaneous staining with FITC-conjugated mouse-anti-human HIV-1-p24 mAb and PE-conjugated mouse-anti-human HO-1 mAb. The data are representative of three independent experiments.