European Journal of Pharmacology 780 (2016) 142–147

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Molecular and cellular pharmacology

Inhibition of myeloperoxidase-mediated oxidative damage by nitrite in SH-SY5Y cells: Relevance to neuroprotection in neurodegenerative diseases

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ARTICLE INFO

Article history:
Received 12 December 2015
Received in revised form
14 March 2016
Accepted 23 March 2016
Available online 25 March 2016

Keywords:
MPO
Nitrite
Anti-oxidant
Oxidative/nitrative stress
Neurochemistry

ABSTRACT

Myeloperoxidase (MPO) and MPO-catalyzed hypochlorous acid (HOCl) is elevated in many neurodegenerative diseases, and lead to severe tissue injuries. Nitrite (NO_2^-) is a widespread inorganic molecule that has recently been proposed as a direct NO donor to exert antioxidant properties in vivo and vitro. Since NO_2^- and MPO (and/or HOCl) were important mediators in brain function and disease, we investigated the effects of NO_2^- on MPO-mediated damage to human neuroblastoma SH-SY5Y cells. Here, we showed that exposure of SH-SY5Y cells to MPO (or HOCl) resulted in a significant loss in viability, ATP and glutathione levels, and treatment of neuronal cells with NO_2^- substantially attenuated MPO (or HOCl)-dependent cellular toxicity. The protective effects of NO_2^- on MPO (or HOCl)-induced cytotoxicity were because that (1) NO_2^- at high concentrations competed effectively with Cl^- for MPO, thus limiting OCl^- production by the enzyme; (2) HOCl was removed by reacting with NO_2^-, forming less damaging compound; (3) NO_2^- significantly inhibited MPO-mediated inactivation of brain protein (enolase) and protein oxidation. Therefore, NO_2^- could show novel protective effects in some neurodegenerative diseases by preventing MPO-mediated oxidative damage.

1. Introduction

Mounting evidence points to an important role of oxidative damage in the pathogenesis of neurodegenerative disorders including Alzheimer's disease (AD) (Zhu et al., 2007). The underlying mechanism is believed to involve oxidative damage to various cell components, including proteins, lipids, and nucleic acids, because of overproduction of free radicals, leading to impairment of cellular functions and ultimately cell death (Maki et al., 2009; Rauk, 2009, Yap et al., 2007).

Epidemiological evidence has suggested an elevated incidence of AD associated with increased myeloperoxidase (MPO) expression (Green et al., 2004; Whiteman et al., 2005). MPO reacts with H_2O_2 to oxidize chloride, producing the potent oxidant, hypochlorous acid (HOCl). The physiological role of MPO is to kill invading bacterial and fungal pathogens using HOCl (Arnhold and Flemmig, 2010; van der Veen et al., 2009; Vlasova et al., 2012; Xu et al., 2009). During inflammation, however, the excessive reactive intermediates it spawns may also oxidize host bio-molecules (van der Veen et al., 2009). MPO also oxidizes nitrite (NO_2^-) and tyrosine to produce nitrogen dioxide (NO_2) and tyrosyl radicals that cause protein tyrosine nitration (Lu et al., 2014b; Pacher et al., 2007; Yap et al., 2007). Furthermore, elevated levels of nitrated and oxidized proteins have been detected in AD brain (Butterfield et al., 2006, 2007). Thus, MPO and its product HOCl cause the extensive oxidative damage observed in brain sections of AD, contributing to neuronal dysfunction and memory loss (Maki et al., 2009; van der Veen et al., 2009; Yap et al., 2007). Therefore, it is important to identify the pathways for MPO detoxicity in vivo.

NO_2^- is one of the major end products of NO metabolism, and from the daily consumption (Lu et al., 2014a; Lundberg et al., 2009; Pacher et al., 2007; Whiteman et al., 2002, 2003). It has been found that endogenous levels of NO_2^- in the brain may reach the micromolar range, and significantly lower NO_2^- levels are reported in the brains of AD patients (~2.7 μM in healthy brain, ~1.7 μM in AD brain) (Hensley et al., 1998). Recently, NO_2^- has emerged as an endogenous signaling molecule with potential therapeutic implications for some diseases. It is now clear that as a direct NO donor, NO_2^- can act as an anti-oxidant and a promising therapeutic agent to protect brain against in vivo ischemia-reperfusion injury through mediating NO homeostasis (Jung et al., 2006; Lundberg et al., 2009). Nitrite reduction to NO is catalysed

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http://dx.doi.org/10.1016/j.ejphar.2016.03.042
0014-2999/

by various enzymatic (such as deoxyhemoglobin, xanthine oxidase) and non-enzymatic (such as acidic disproportionation) pathways, which may be of therapeutic value (Lundberg et al., 2009).

In the present study, we showed that NO_2^- prevented MPO from causing injuries to neuronal cells and that NO_2^- directly protected the brain cells against useful but potentially harmful HOCl, which were different from the recent therapeutic role as a NO donor and not focus on previous studies. The potential antioxidant action of this new neuromodulator may represent a novel pathway for a reduction of risks associated with neurodegenerative diseases.

2. Materials and methods

2.1. Materials

Myeloperoxidase (MPO) from human leukocytes, glucose, glucose oxidase (GO), taurine, sodium hypochlorite (NaOCl), enolase from *bakers yeast*, sodium nitrite ($NaNO_2$), 2, 4-dinitrophenylhydrazine (DNPH), rabbit polyclonal antibody against dinitrophenol (DNP) and 3-nitrotyrosine (3-NT) were purchased from Sigma. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) was purchased from Gibco BRL. All solvents and other reagents were of the highest purity commercially available.

Absorption spectra of MPO were recorded by the use of a spectrophotometer (Hitachi U-3310, Japan) at room temperature.

2.2. Cell culture and cell viability determination

2.2.1. Exposure of cells to HOCl and NO_2^-

The human neuroblastoma SH-SY5Y cell line was chosen as an established cell model of oxidative stress to neuronal cells (Whiteman et al., 2005). Cells were washed twice with PBS and once with DMEM, and different concentrations of $NaNO_2$ were added for 2 min. Then, HOCl was added and the cells were incubated further for 10 min. Cell viability was quantified by using MTT assay (Whiteman et al., 2002, 2003).

2.2.2. Exposure of cells to MPO system and NO_2^-

SH-SY5Y cells were cultured in DMEM containing NaCl (100 mM), glucose (5.6 mM) (Xu et al., 2009). Different concentrations of $NaNO_2$ were first added to cells for 2 min. Then, MPO (1 U/mL) was added and incubated for 10 min. Subsequently, the MPO reaction was initiated by adding GO (10 mU/mL) to generate H_2O_2, and cells were maintained for 2 additional hours. After that, cellular viability was measured by using MTT assay.

2.3. Effect of NO_2^- on chlorinating activity of MPO

The chlorinating activity of MPO was measured by taurine chloramine assay as described previously (Vlasova et al., 2012). In the presence or absence of NO_2^-, MPO (0.5 μM) was added to a mixture of NaCl (100 mM), taurine (1 mM) and H_2O_2 (500 μM) in 100 mM PBS, pH 7.0 and incubated for 10 min at room temperature. Then, the amount of taurine chloramines formed as the result of the reaction between taurine and HOCl was measured (Vlasova et al., 2012). After the reaction of NO_2^- with HOCl, NO_2^- remaining in solution was measured by Griess method.

2.4. Oxidative and nitrative modifications of enolase by MPO-H_2O_2-NO_2^-

Enolase (0.1 mg/mL) was treated with MPO (1 μM)-H_2O_2 (0.5 mM)-NaCl (100 mM) in the presence of different concentrations of NO_2^- in 100 mM PBS at 37 °C for 30 min. Aliquots were taken for blot analysis of tyrosine nitration, protein carbonyl and determination of enzyme activity (Lu et al., 2014a, 2014b).

2.5. Statistical analysis

The results were presented as the means ± S. D. of at least three experiments. One-way ANOVA was performed for statistical analyses, and $P < 0.05$ was considered significant.

3. Results

3.1. NO_2^- protected cells against injuries caused by MPO activity

We first used human neuroblastoma SH-SY5Y cells to confirm that MPO caused severe cell injuries or cell death by producing OCl^-. To mimic H_2O_2 concentration in the brain from AD patients (Whiteman et al., 2005; Xu et al., 2009; Yap et al., 2007), we

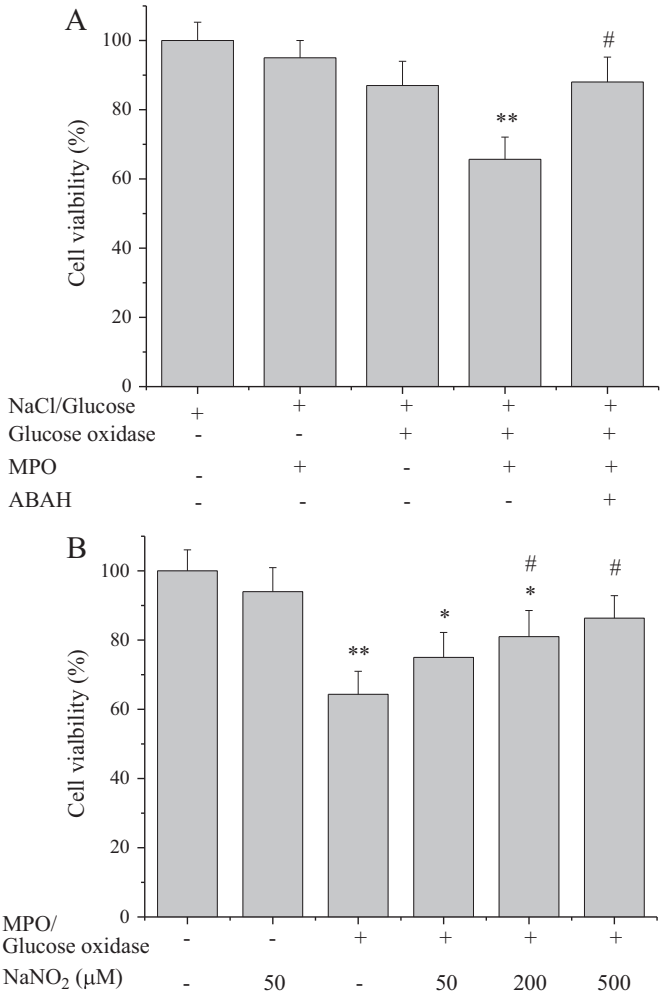

Fig. 1. Cytotoxicity of MPO (A) and the protective effects of $NaNO_2$ on MPO cytotoxicity (B). SH-SY5Y cells were cultured in DMEM containing NaCl (100 mM), glucose (5.6 mM), pH 7.0. Different concentrations of $NaNO_2$ were added to cells for 2 min. In the absence or presence of MPO inhibitor ABAH (100 μM), MPO (1 U/mL) and glucose oxidase (10 mU/mL) were then added. The Blank values were set to 100%, to which other values were compared (Values are means ± S.D. of three independent determinations, A: $^{**}P < 0.01$, compared to Blank group (no MPO/GO/$NaNO_2$ added); $^{\#}P < 0.05$, compared to MPO/G/GO/NaCl-treated group; B: $^{*}P < 0.05$, $^{**}P < 0.01$, compared to Blank group (no MPO/GO/$NaNO_2$ added); $^{\#}P < 0.05$, compared to MPO/G/GO/NaCl-treated group).