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Proton pump inhibitors exert anti-inflammatory effects and decrease human microglial and monocytic THP-1 cell neurotoxicity

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ABSTRACT

To explore whether proton pump inhibitors (PPIs) possess anti-inflammatory effects on microglia, we investigated the effect of lansoprazole (LPZ) and omeprazole (OPZ) on the toxic action towards SH-SY5Y neuroblastoma cells of supernatants from human microglia and THP-1 cells stimulated by lipopolysaccharide combined with interferon-γ. In addition, we studied the effect of LPZ and OPZ on the THP-1 cell production of the pro-inflammatory cytokines tumor necrosis factor (TNF)-α and interleukin (IL)-6 using enzyme-linked immunosorbent assays. We found that both PPIs had a protective effect on the toxicity of supernatants and that there was a synergism of this effect with *S*-ibuprofen (IBP), a typical non-steroidal anti-inflammatory drug (NSAID). A similar protective effect of LPZ was observed with supernatants from stimulated human microglia. We also found that both PPIs significantly reduced the TNF-α secretion from stimulated THP-1 cells in a concentration dependent manner and that there was a trend towards such reduction of IL-6. These results indicate that PPIs possess anti-inflammatory effects and can decrease human microglial and monocytic neurotoxicity. They suggest that PPIs combined with NSAIDs may be effective in the treatment of a broad spectrum of neurodegenerative diseases associated with activated microglia.

Introduction

Lansoprazole (LPZ) and omeprazole (OPZ) are proton pump inhibitors (PPIs) which suppress the H^+/K-ATPase enzyme present in parietal cells of gastric mucosa. As a result, they strongly inhibit gastric acid secretion. In general, PPIs are well tolerated with few adverse effects. Therefore, PPIs are widely used for the treatment of gastric and duodenal ulcers as well as reflux esophagitis. In addition, PPIs are used for the treatment of gastrointestinal complications caused by non-steroidal anti-inflammatory drugs (NSAIDs), including ulcers (for reviews see Boparai et al., 2008; Isomoto et al., 2007). Besides such anti-secretory effects, PPIs have some anti-inflammatory actions. Specifically, LPZ and OPZ have been reported to attenuate human neutrophil adherence to endothelial cells *in vitro* by inhibiting the expression of intercellular adhesion molecule-1, vascular adhesion molecule-1 and IL-8 (Handa et al., 2006; Yoshida et al., 2000). LPZ and OPZ were also reported to inhibit the production of oxygen-derived free radicals by activated human neutrophils *in vitro* (Suzuki et al., 1995, 1996).

Inflammatory processes, including microglial activation, are closely associated with the pathogenesis of a broad spectrum of neurodegenerative diseases (for reviews see McGeer and McGeer, 2004; Mena et al., 2008; Qian and Flood, 2008; Whitton, 2007). Alzheimer's disease (AD) is a prominent example (Akiyama et al., 2000). Activated microglia play crucial roles through the production of potentially neurotoxic molecules such as pro-inflammatory cytokines and superoxide radicals (Hashioka et al., 2007a,b; Meda et al., 1995). It is tempting to speculate that PPIs may be effective in the treatment of neurodegenerative diseases due to their anti-inflammatory activity.

To evaluate the potential of PPIs to treat neuroinflammatory disorders, we investigated the effect of two PPIs (LPZ and OPZ) on the toxicity of activated human microglia and THP-1 human monocytic cells towards SH-SY5Y human neuroblastoma cells. In addition, we studied the effect of LPZ and OPZ on the THP-1 cell production of the pro-inflammatory cytokines tumor necrosis factor (TNF)-α and interleukin (IL)-6. Since we have previously shown that the classical NSAID *S*-ibuprofen (IBP) reduces human microglial and THP-1 cell neurotoxicity (Klegeris et al., 1999, 2004), we also tested the effects of the combination of PPIs and IBP on THP-1 cell neurotoxicity and their cytokine production.

Materials and methods

Chemicals and reagents

Human recombinant interferon (IFN)-γ was purchased from PeproTech (Rocky Hill, NJ, USA). LPZ, OPZ, bacterial lipopolysaccharide (LPS, from *E. coli* 055:B5) and 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma (St.

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Louis, MO, USA). IBP was obtained from Biomol (Plymouth Meeting, PA, USA). LPZ and OPZ were initially dissolved in dimethyl sulfoxide (DMSO). The final concentration of DMSO in tissue culture medium was less than 0.01%. At this concentration, DMSO had no effect on cell viability.

Cell cultures

The human monocytic THP-1 cell line was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The human neuroblastoma SH-SY5Y cell line was a gift from Dr. Robert Ross (Department of Biological Sciences, Fordham University, Bronx, NY). These cells were grown in Dulbecco's modified Eagle medium (DMEM), nutrient mixture F12 Ham (DMEM-F12) supplemented with 10% fetal bovine serum (FBS) (Invitrogen Canada, Burlington, ON, Canada) and penicillin (200 U/ml)/streptomycin (200 μg/ml) (Invitrogen Canada). Both cell lines were used without initial differentiation. Adult human dopaminergic neurons are not available and cultures of any human neurons are technically difficult to prepare. The low success rate and cell yield does not lend itself to large scale studies (Brewer et al., 2001). Therefore, human neuroblastoma SH-SY5Y cells were used to model human neurons even though they do not display fully characteristics of adult neurons (Pahlman et al., 1984; Ross et al., 1981).

Human microglia were obtained from epileptic patients undergoing temporal lobe surgery. The specimens were from normal tissue overlying the epileptic foci. The use of human brain materials was approved by the Clinical Screening Committee for Human Subjects of the University of British Columbia. Specimens were washed with DMEM-F12 and chopped into small (<2 mm^3) pieces using a sterile scalpel. The fragments were incubated in 10 ml of 0.25% trypsin solution at 37 °C for 15 min. Subsequently DNase I (from bovine pancreas, Pharmacia Biotech, Baie d'Urfe, PQ, Canada) was added to reach a final concentration of 50 μg/ml. Tissues were incubated for an additional 5 min at 37 °C. The cell suspension was diluted with 10 ml of DMEM-F12 with 10% FBS and gently triturated using a 10 ml pipette with a wide mouth. After centrifugation at 275 *g* for 10 min, the cell pellet was resuspended in the DMEM-F12 with 10% FBS, triturated several times, and passed through a 100 μm nylon cell strainer (Becton Dickinson, Franklin Lakes, NJ, USA). The cell suspension was then centrifuged once more (275 *g* for 10 min), cells were resuspended into 10 ml of DMEM-F12 with 10% FBS containing penicillin/streptomycin and plated onto uncoated 10 cm tissue culture plates (Becton Dickinson). Plates were placed in a humidified 5% CO_2, 95% air atmosphere at 37 °C for 2 h. Microglial cells were separated by allowing them to adhere to the tissue culture plates. The non-adherent cells with myelin debris were discarded by replacing the cell medium in the plates. After 5 to 7 days in culture, microglial cells were detached from the plates by trypsinization (0.25% trypsin with EDTA, Invitrogen Canada) and then resuspended into DMEM-F12 containing 5% FBS and used for assays. Purity of microglial cultures was estimated by immunostaining with antibodies against CD68, which stains microglia as well as macrophages, and against glial fibrillary acidic protein (GFAP). Both these antibodies were used to ensure an accurate estimate. Thus, in our cultures, it was possible to demonstrate that more than 95% of the cells were CD68 positive with less than 5% cells being stained by the anti-GFAP antibody.

Toxicity of microglia and THP-1 cells towards SH-SY5Y neuroblastoma

Human monocytic THP-1 cells were seeded onto 24-well plates at a concentration of 5×10^5 cells per well in 1 ml of DMEM-F12 medium containing 5% FBS, and human microglial cells were used at a concentration eight times less. The cells were incubated in the presence of various drugs or corresponding vehicle solutions for 15 min prior to the addition of activating stimulants (0.5 μg/ml of LPS combined with 150 U/ml of IFN-γ). The combination of 0.5 μg/ml LPS and 150 U/ml IFN-γ has previously been shown to induce significant neurotoxicity in the supernatants of microglial as well as THP-1 cells (Klegeris and McGeer, 2000; Klegeris et al., 2004, 2008). After 24-h incubation of THP-1 cells at 37 °C, 0.4 ml of cell-free supernatant was transferred to each well containing SH-SY5Y cells. At this time point, the THP-1 cell viability was measured by the MTT assay. Microglial cells were incubated for 48 h after stimulation with LPS plus IFN-γ to induce sufficient cytotoxicity. After such stimulation, 0.4 ml of cell-free supernatant was transferred to each well containing SH-SY5Y cells. At this time point, the microglial cell viability was also measured by the MTT assay. SH-SY5Y cells had been plated 24 h earlier at a concentration of 2×10^5 cells in 0.4 ml of DMEM-F12 medium with 5% FBS. After 72 h of incubation at 37 °C, evaluation of surviving cells was performed by the MTT assay. The following procedures were used to establish that PPIs did not neutralize neurotoxins in the supernatants and did not act on SH-SY5Y cells directly. Supernatants from THP-1 cells activated by LPS/IFN-γ for 24 h in the absence of drugs were collected and the drugs under study (i.e., 5 μM LPZ, 10 μM OPZ, 5 μM LPZ plus 1 μM IBP, and 10 μM OPZ plus 1 μM IBP) were added into the supernatants just before applying them to the SH-SY5Y cells. We also added these drugs to the SH-SY5Y cell culture directly. After 72-h incubation at 37 °C, the SH-SY5Y cell viability was measured by the MTT assay.

Cell viability assays: MTT assay

The MTT assay was performed as described previously (Klegeris and McGeer, 2000; Klegeris et al., 2004, 2008). The viability of SH-SY5Y cells was determined by adding MTT to the SH-SY5Y cell cultures to reach a final concentration of 1 mg/ml. Following 1 h incubation at 37 °C, the dark crystals formed were dissolved by adding to the wells an equal volume of sodium dodecyl sulfate/N, N-dimethylformamide (SDS/DMF) extraction buffer (20% SDS, 50% DMF, pH 4.7). Subsequently, plates were warmed overnight at 37 °C in order to dissolve aggregates of lysed cells. Optical density at 570 nm was measured by transferring 100 μl of aliquots to 96-well plates and the values were recorded by using a microplate reader. The viability of SH-SY5Y cells exposed to supernatants from microglia or THP-1 cells was determined as a percentage of the value obtained from cells incubated in fresh medium only.

The residual value for 0% cell survival was determined by lysing the cells with 1% Triton X-100. The viabilities of THP-1 cells and microglial cells were also measured by the same method.

Measurement of TNF-α and IL-6: enzyme-linked immunosorbent assay (ELISA)

THP-1 cells were seeded onto 24-well plates at a concentration of 5×10^5 cells per well in one ml of DMEM-F12 medium containing 5% FBS. The cells were incubated in the presence of various drugs for 15 min prior to the addition of activating stimulants (0.5 μg/ml of LPS combined with 150 U/ml of IFN-γ). After 24-h incubation at 37 °C, 100 μl of cell-free supernatants were assayed for TNF-α and IL-6 accumulation. The concentrations of TNF-α and IL-6 were measured by using corresponding ELISA development kits supplied by Pepro-Tech. The assays were based on the quantitative sandwich enzyme immunosorbent technique and carried out according to the manufacturer's protocols.

Statistics

All values are expressed as the means ± standard error of the mean (S.E.M.). Comparisons were made with a one-way analysis of variance followed by the post hoc Tukey–Kramer test using StatView 5.0