In vitro effects of *Helicobacter pylori*-induced infection in gastric epithelial AGS cells on microglia-mediated toxicity in neuroblastoma SH-SY5Y cells

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Abstract. Objective: To investigate the in vitro effects of *H. pylori*-conditioned medium (HCM) from gastric epithelial AGS cell cultures on microglia and neuronal cells. Material: *H. pylori*, human gastric epithelial AGS cells, microglia-like BV-2 cells and human neuroblastoma SH-SY5Y cells. Treatment: Treated AGS cells with *H. pylori* at ratios from 1:100 to 1:900 for 24 h. Cultured BV-2 cells and SH-SY5Y cells were treated with HCM from AGS cell cultures. Methods: Cell viability was measured by a quantitative colorimetric assay with MTT. Nitric oxide (NO) was determined by using Griess reagent. IL-8 was measured by an enzyme-linked immunosorbent assay. Protein expressions were revealed by western blot analysis. Results: *H. pylori* increased IL-8, NO, COX-2 and gp91phox in AGS cell cultures. When BV-2 cells were co-cultured with AGS cells, HCM increased COX-2, gp91phox, iNOS and NO of BV-2 cells. HCM also enhanced the degradation of IkBa in BV-2 cells. HCM up-regulated expression of nNOS, COX-2, and gp91phox of SH-SY5Y cells co-cultured with BV-2 cells. Particularly, the decrease of cell viability of SH-SY5Y induced by HCM was dependent on the presence of BV-2 cells. Conclusions: *H. pylori*-induced infection induces microglia-mediated inflammation and neurotoxicity. The present results suggest that microglia play a critical role in HCM-induced toxicity of neuronal SH-SY5Y cells.

Key words: *Helicobacter pylori* – Inflammation – Microglia – Neurotoxicity.

Introduction

Helicobacter pylori (*H. pylori*), a gram-negative bacteria, plays a causal role in a variety of gastric diseases including chronic gastritis, peptic ulcer and gastric cancer [1, 2]. *H. pylori* induces inflammation-associated gene expression in gastric epithelial cells, including activation of nuclear factor kappa B (NF-κB), enhanced expression of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), and production of inflammatory cytokines [3, 4]. A number of studies have shown increased levels of cytokines including interleukin (IL)-1α, IL1-β, IL-6, IL-8 and tumor necrosis factor (TNF)-α in gastric mucosa of patients with *H. pylori* infection. IL-8 secreted by gastric epithelial cells are likely to be important host mediators inducing neutrophil migration to the site of infection and therefore may be important in the regulation of inflammatory and immune processes in response to *H. pylori* [5, 6]. NF-κB may play a novel role in expression of IL-8 and COX-2 in *H. pylori*-induced gastric inflammation [7]. Reactive oxygen species (ROS) and *H. pylori* have been identified as pathogenic factors in several gastrointestinal disorders [8]. Exposure of gastric epithelial cells to *H. pylori* results in an inflammatory reaction with production of intracellular ROS and NO, and enhances membrane damage [8]. Study suggests nicotinamide adenine dinucleotide phosphate (NADPH) oxidase might be involved in ROS production in *H. pylori* infection.
Recent research on the nerve-immune interactions demonstrates inflammation-associated neurodegeneration which can lead to motility related problems in diabetes. Changes in the inhibitory and excitatory enteric neurons are described, highlighting the loss of inhibitory neurons in early diabetic enteric neuropathy [10]. Many of the functions of the gastrointestinal tract are subject to neural regulation by the enteric nervous system (ENS) and its extrinsic connections. Information input processed by the ENS is derived from local sensory receptors, the central nervous system, and immune/inflammatory cells including mast cells. Inflammation increases excitability of intrinsic primary afferent neurons and alters synaptic transmission to interneurons and motor neurons in the ENS [11, 12]. Inflammation induced changes to the ENS and mucosal enteroendocrine cells [13]. COX-2 contributes to dysmotility and enhanced excitability of myenteric afterhyperpolarizing neurones [14]. H. pylori infection induces functional and morphological changes in the gastric neural circuitry [15].

Inflammation-mediated neurotoxicity has been implicated in numerous neurodegenerative diseases [16]. COX-2 is a critical enzyme in the inflammatory response [17]. COX-2 functions as a cellular factor which induces superoxide-mediated cell death in primary cortical neurons [18]. Oxidative/nitrosative stress generally describes a condition in which ROS and reactive nitrogen species reach levels where cellular antioxidant defenses are inadequate or overwhelmed [19]. Increasing evidence suggests that ROS and reactive nitrogen species participate in neurodegenerative diseases [20, 21]. The phagocyte NADPH oxidase becomes activated during phagocytosis...