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Microbes and Alzheimer' disease: lessons from *H. pylori* and GUT microbiota

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Abstract. – OBJECTIVE: the role of microbes and chronic inflammation in the pathogenesis of Alzheimer' disease (AD) has been postulated by many authors. On the other hand, several studies have reported the main role of *H. pylori* infection and/or GUT microbiota alteration in promoting chronic inflammation, thus possibly influencing both occurrence and evolution of AD. In this article, we analyze the most important and recent studies performed on this field both on humans and animals and provide possible pathogenic explanations.

RESULTS: all main and most recent animal, human, epidemiological and in-silico studies, showed a role of *H. pylori* and/or dysbiosis in AD, mostly through the promotion of systemic chronic inflammation and/or by triggering molecular mimicry mechanisms. In particular, *H. pylori* infection seems to be related to a poorer cognitive performance.

CONCLUSIONS: Indeed, bacteria have been shown to affect neurodegeneration by promoting inflammation, inducing molecular mimicry mechanisms and accumulation of Aβ into the brain. These findings open the way for *H. pylori* eradicating trials and/or GUT microbiota remodulating strategies. Therefore, further studies are now needed in order to test whether antibiotics, pre and/or probiotics may exert a beneficial effect in the prevention of AD.

Key Words:
H. pylori, Microbiota, Alzheimer' disease, Molecular mimicry, Inflammation, Amyloid.

Introduction

The role of microbes as a possible cause of acute and chronic diseases has been investigated by many researchers for a long time; indeed, bacteria and viruses have been demonstrated to affect organs even far from the primary site of infection, especially through translocation and/or molecular mimicry mechanisms[1].

Gut microbiota is a complex system of microorganisms showing an unequal distribution from the mouth to the anus and covering several biological functions[2]. Overall, it is composed by viruses, bacteria, yeasts and protozoa, all living in relative harmony, but more information is now available concerning bacterial community[3]. On this subjects, two main dominant families have been recognized, such as *Firmicutes* and *Bacteriodetes*, along with other less represented species, including C*lostridia*, *Proteobacteria,* and *Actinobacteria*[4]. *Helicobacter* species are classified among *Proteobacteria* and include a wide range of subspecies colonizing stomach, gut, and bile[5]. *H. pylori* is the most known species and is the main cause of gastritis, peptic ulcer and gastric cancer in humans[6], while some authors place *H. pylori* among the GUT microbiota species, since in some specific conditions, it may act as a symbiont. This is the case of allergic asthma and/or atopic dermatitis, both showing a negative correlation with *H. pylori* infection, possibly due to the stimulation of the Th1 and the suppression of the Th2 immune response, thus promoting inflammation but alleviating allergic reactions[7,8].

GUT microbiota composition may vary among healthy and unhealthy individuals[9,10]. In normal subjects, GUT microbiota lives in *eubiosis*, meaning maintaining diversity, richness and relative abundance. In this way, GUT microbiota and host co-exist in a cooperative systemic aggregation model, both contributing to regulation the barrier effect, metabolism, immunocompetence and tolerance and influencing synthesis of many substan-

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ces including neurotransmitters, drug metabolism and even behavior conditioning[11,12]. However, all factors able to alter *eubiosis*, such as the massive use of antibiotics and/or anti-acids, the impairment of the immune system or the alteration of the integrity of the GI mucosal barrier, are able to produce a pathological condition called *dysbiosis*, which in turn is strongly related to the occurrence of both GI or extra-GI diseases, including neurological disorders, through the promotion of a pro-inflammatory status[13]. This is why some researchers explored the possibility that *H. pylori* infection and/or intestinal dysbiosis, persisting for decades, may possibly influence both occurrence and development of Alzheimer disease (AD). In this article, we analyze the most important and recent studies performed on this field and provide possible pathogenic explanations.

H. pylori and Alzheimer' disease

Chronic *H. pylori* infection might influence the development and course of AD via a wide variety of mechanisms. A cross-sectional study performed by Beydoun et al[14], who analyzed data from the US national health and nutrition examination survey, explored the correlation between *H. pylori* positivity and cognitive performance among US adults. Results showed a poorer performance of *H. pylori*-positive 60-90 years old subjects, concerning verbal memory test compared to negative. Moreover, 20-59 years old infected non-Hispanic black and women performed worse on serial digits learning total errors compared to uninfected. This was a clear demonstration that *H. pylori* may, in some way correlate with cerebral function even if its causative role is still to be demonstrated. In a recent study, Bu et al[15] tested the hypothesis that AD may be associated to an infectious burden (IB) sustained by viruses (CMV and HSV-1) and bacteria, including *Borrelia burgdorferi*, *Chlamydia pneumoniae,* and *H. pylori*. They tested 128 AD patients and 135 healthy controls for serological evidence of those microorganisms, reporting a significant association between IB and AD. Concerning pathogenic mechanisms, they demonstrated higher levels of serum beta-amyloid protein (Aβ), such as Aβ40, Aβ42 and total Aβ and cytokines, such as TNFα, IL-1β, and IL-6 in subject positive to 4-5 infectious agents compared to uninfected. Similarly, Roubaud Baudron et al[16] examined both clinical and biological data of 53 patients with AD, testing the suggestion that *H. pylori* may alter the cognitive status by increasing inflammation. Results showed a lower mini-mental state examination (MMSE) score (p=0.017), higher plasma IL1β levels (p=0.025) and increased gastric atrophy (p=0.020) in infected subjects compared to uninfected a higher cognitive impairment in H. pylori-positive AD patients. In a recent study, Wang et al[17] explored the possible correlation between *H. pylori* infection and the abnormal hyperphosphorylation of microtubule-associated protein tau, strongly implicated in AD pathogenesis. Interestingly, they found that *H. pylori* increase tau-hyperphosphorylation, which in turn is attenuated by the GSK-3 inhibitor, thus providing evidence of a potential role in the promotion of AD and opening the way for new *H. pylori* eradicating trials. Those data are also supported by animal studies; the same authors, in a recent trial[18], tested the effect of *H. pylori* on cognitive function and Aβ production in rats. They concluded that soluble surface fractions of *H. pylori* can impair cognitive functions and promote Aβ42 formation, thus interfering with synaptic functions. More recently, Boziki et al[19] suggested that *H. pylori* may affect AD by interacting with Galectin-3, a glycan-binding protein implicated in several physiologic and pathologic processes, including cell signaling, proliferation, and migration as well as in the stimulation of immune response.

Finally, a recently published review article[20] suggest different main mechanisms that inclu-

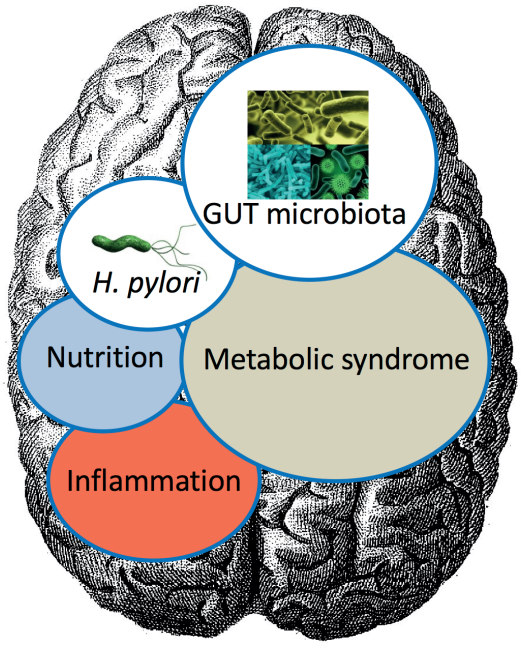

Figure 1. Main mechanisms by which *H. pylori* and dysbiosis may affect the development and evolution of AD.

de the possibility of the bacterium to enter in contact with the brain through the oral-, nasal-, olfactory-pathway or through the disrupted blood-brain barrier, thus triggering the process of neurodegeneration.

Based on all those data, we may speculate that *H. pylori* may influence AD occurrence and development by promoting chronic inflammation with all the above-mentioned consequences. However, no interventional studies aiming to demonstrate an eventual causative role of dysbiosis has been carried out so far.

GUT microbiota and Alzheimer' disease in experimental models and in specific human disease aspects

In a recent animal study, Park et al[21] investigated differences in extracellular vesicles (EVs), containing bacterial genomic DNA fragments and other proteins secreted and attributable to GUT microbiota species, between normal mouse and a mouse model of AD (Tg-APP/PS1). Of note, they described a significant difference both at the phylum level, such as increasing of *Firmicutes* and decrease of *Proteobacteria* and *Bacteriodetes* and at the genus level, including increasing of *Aerococcus, Jeotgalicoccus, Blautia, Pseudomonas, Clostridial* and *Ruminococcaceae* and decreasing of *Lactobacillus* and *Corynebacterium* in Tg-APP/PS1 mice compared to wild-type. Similarly, Wu et al[22] reported how enterobacteria infection in a Drosophila model of AD may exacerbate disease progression by increasing levels of inflammatory cytokines.

The possible role of polyphenol on AD progression has also been investigated by Wang et al[23], who administered grape seed polyphenol extract to rats, in order to verify bioavailability of 12 phenolic acids known to be generated by GUT microbiota metabolism. Results showed a significant increase of two phenolic acids, such as 3-(3'-hydroxyphenyl)propionic acid and 3-hydroxybenzoic acid, in the brain of treated animals. Moreover, they also demonstrated that 3-(3'-hydroxyphenyl) propionic acid may interfere with the assembly of Aβ peptide into a neurotoxic β-amyloid aggregate, which was shown to play a role in the development of AD. The authors have then concluded that the protective effect of polyphenols on AD is mediated by GUT microbiota species, which in turn may be a future target for AD prevention. Similarly, Yuan et al[24] have studied the effect of pomegranate extract, which was previously shown to exert a protective effect in patients with AD; they reported that its neuroprotective effect is due to the effect of urolithins, a metabolite produced through the action of GUT microbiota species.

Scholars have clearly remarked the role of bacteria in the pathogenesis of AD; on this subject, Zhao et al[25] reported very high levels of bacterial lipopolysaccharide (LPS) in lysates from hippocampus and superior temporal lobe neocortex of AD brains. They have then speculated a possible role of some bacterial components of the GUT microbiota in this pathological process. Similarly, Cattaneo et al[26] measured inflammatory cytokine levels in 40 cognitively impaired patients, 33 with no evidence of brain amyloidosis and in 10 healthy controls and matched data with the load of specific bacterial species of the GUT microbiota. Results showed that the pattern of increased *Escherichia/Shighella* and reduced *E. rectale* was associated with a higher peripheral inflammatory state, cognitive impairment, and brain amyloidosis.

The potential role of GUT microbiota in the pathogenesis of AD has also been demonstrated by in-silico studies; Negi et al[27] tested the molecular mimicry hypothesis, based on the assumption that peptide similarity between host and microorganisms may be possibly implicated in AD. They found a correlation between increased *Firmicutes* and *Proteobacteria* phyla and different diseases, including AD, type 2 diabetes mellitus, chronic kidney failure, and chronic obstructive pulmonary disease. The same mechanisms have been considered by Friedland[28], who identified the interactions among cross-seeding of amyloid misfolding and oxidative stress induced by some bacteria composing the GUT microbiota; they deliberated that bacterial amyloid may then induce molecular mimicry mechanisms involving the brain and promoting neurodegeneration. Xu et al[29] performed a study by analyzing a vast amount of biomedical data, including genetic, microbial and protein pattern, correlating them with commonalities with AD. They have then identified metabolites significantly associated with various aspects of AD, including trimethylamine N-oxide, a gut microbial metabolite of dietary meat and fat, which in turn contribute to AD development. Nutrients may indeed play in favor or against AD generation and progression, but all related mechanisms are mediated by the GUT microbiota, as reported by Pistollato et al[30]. On the other hand, the relationship among nutrition, metabolic syndrome, type

2 diabetes mellitus and AD has been described by many authors, all conditions associated with dysbiosis and increased inflammatory state[31]. Similarly, Kohler et al[32] proposed a possible role of leaky gut in the pathogenesis of AD. Based on his assumption, dysbiosis may in some subjects promote leaky gut with abnormal bacterial translocation, possibly increasing inflammation and accumulation of Aβ. On this subject, Chen et al[33] reported an association between irritable bowel syndrome (IBS) and increased risk of dementia by studying 298 patients with IBS and 192 controls. Interestingly, IBS is strongly associated with dysbiosis and leaky gut, thus reinforcing the role of GUT microbiota in neurodegeneration.

Conclusions

The role of chronic inflammation as a risk factor for AD development has been clearly established, as well as the influence of nutrition and metabolic syndrome[34]. On the other hand, all those conditions have been demonstrated to be associated with *H. pylori* infection and/or an imbalance of the GUT microbiota composition (Figure 1). Notably, *H. pylori* infection has been shown to alter gastric pH, thus influencing both gastric and GUT microbiota composition and promoting dysbiosis[35,36], which in turn is now considered as a possible key point for AD occurrence and development. Indeed, bacteria have been shown to affect neurodegeneration by promoting inflammation, inducing molecular mimicry mechanisms and accumulation of Aβ into the brain. Those effects have been reported by animal, human, epidemiological and in-silico studies and open the way for *H. pylori* treatment and/or GUT microbiota remodulation strategies. Since *H. pylori* eradication may be a possible cause of dysbiosis, through the action of antibiotics[37] concomitant treatment with probiotics, during the course of the eradicating treatment, should be provided[38]. As a consequence, further interventional studies aiming to demonstrate a causative role of dysbiosis need in order to test whether antibiotics, pre and/or probiotics may exert a beneficial effect in patients with AD.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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