NeuroImage: Clinical 20 (2018) 793–799

Contents lists available at ScienceDirect

NeuroImage: Clinical

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

Neurotransmitter alterations in the anterior cingulate cortex in Crohn's disease patients with abdominal pain: A preliminary MR spectroscopy study

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ARTICLE INFO

Keywords:
Brain-gut axis
Crohn's disease
Glutamate
Metabolite
Pain
Spectroscopy

ABSTRACT

Purpose: Crohn's disease (CD) has been known to cause both abdominal pain alongside functional and structural alterations in the central nervous system (CNS) in affected patients. This study seeks to determine the alternations of metabolites in the bilateral anterior cingulate cortex (ACC) of CD patients with abdominal pain by using proton magnetic resonance spectroscopy (^{1}H-MRS) to further explore the neural mechanism.

Methods: Sixteen CD patients with abdominal pain and 13 CD patients without abdominal pain, were recruited alongside 20 healthy controls (HCs) for this study. Clinical evaluations, including the 0–10 Visual Analogue Scale (VAS) of pain, Hospital Anxiety and Depression Scale (HADS) and Crohn's Disease Activity Index (CDAI), were evaluated prior to MR scanning. This study selected the bilateral ACC as the region of interest (ROI). The metabolites of the bilateral ACC were quantitatively analyzed by LCModel and Gannet. A independent sample *t*-test and one-way analysis of variance (ANOVA) were performed for statistical analysis. Spearman correlation analyses were performed to examine the relationship between the metabolite levels and clinical evaluations.

Results: The results indicated that CD patients with abdominal pain exhibited significantly higher levels of Glutamate (Glu)/(creatine + phosphocreatine, total creatine, tCr) over CD patients without abdominal pain, and HCs ($p = 0.003$, 0.009, respectively) in the bilateral ACC. The level of (Glutamate + Glutamine, Glx)/tCr of pain CD group was higher than non-pain CD group ($p = 0.022$). Moreover, within the pain CD group, Glu/tCr and Glx/tCr levels correlated strongly with the VAS scores of pain ($\rho = 0.86$, 0.59 respectively, $p < 0.05$). Meanwhile, the results indicates that CD patients with abdominal pain have significantly lower levels of γ-aminobutyric acid plus (GABA+)/tCr ($p = 0.002$) than HCs. To some extent, CDAI demonstrated a trend of negative correlation with GABA+/tCr levels ($p = 0.088$, $\rho = -0.60$).

Conclusion: The neural mechanism of CD patients with abdominal pain in pain processing is tightly associated with neurochemical metabolites. An imbalance in Glu and GABA may play a key role in abdominal pain processing for patients with CD. This mechanism of pain may associate with the intestinal microbiota on the brain-gut axis.

1. Introduction

Crohn's disease (CD) is a chronic, non-specific granulomatous inflammatory disorder which can affect any part of the digestive tract. Geographically, CD is most prevalent in developed, western countries (Molodecky et al., 2012). Moreover, recent epidemiologic studies (Ng et al., 2013) reveal a rapid increase in incidence over recent years. As the chief complaint of CD, abdominal pain causes discomfort in affected patients and significantly decreased quality of life. Pathogenesis of CD in pain processing has not been fully understood, but is commonly believed to be associated with inflammation, visceral hypersensitivity, brain-gut axis dysfunction and psychological abnormalities (Bonaz and Bernstein, 2013; Mayer and Tillisch, 2011). The brain-gut axis (Bonaz and Bernstein, 2013; Collins et al., 2012; Cryan and O'Mahony, 2011) describes a bidirectional communication system involved between the brain and enteric microbiota. Dysfunction of one or more nodes of the

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https://doi.org/10.1016/j.nicl.2018.09.008
Received 1 March 2018; Received in revised form 14 August 2018; Accepted 13 September 2018
Available online 18 September 2018

brain-gut axis would affect CD, which may be related to abnormal pain processing in the central nervous system (CNS).

With regard to the brain-gut axis; a study (Bao et al., 2016) reported that the differences in regional homogeneity (ReHo) of specific brain regions in CD patients with abdominal pain compared with CD patients in remission and without abdominal pain by using resting-state blood oxygenation level dependent functional magnetic resonance imaging (BOLD-fMRI). The specific brain regions included the cingulate cortex, insula, hippocampus, supplementary motor area, temporal pole, and dorsomedial prefrontal cortex. Similarly, in a previous fMRI study (Bernstein et al., 2002), they reported abnormal activity in the anterior cingulate cortex (ACC) and left somatosensory cortex in CD patients during periods of pain. Therefore, it is evident that functional alterations in neurological processing occur in CD patients with abdominal pain.

BOLD-fMRI (Matthews and Jezzard, 2004; Ogawa et al., 1990) was first reported by Ogawa et al. in 1990 and has become a powerful method for detecting brain activity. BOLD-fMRI functions by detecting a local increase in relative blood oxygenation that results from neurotransmitter activity, and thus, indirectly reflects local neuronal firing rates. Furthermore, studies (Attwell et al., 2010; Donahue et al., 2010) have suggested that changes in brain blood oxygen concentration are typically associated with changes in regional neurotransmitters. The neurotransmitters or metabolites can be detected by proton magnetic resonance spectroscopy (^{1}H-MRS), a non-invasive method for detection of biochemical molecules (Jansen et al., 2006), such as γ-aminobutyric acid (GABA), *N*-acetyl-aspartate (NAA), myo-inositol (mIns), choline (Cho), glutamate (Glu), glutamine (Gln), glutamate + glutamine (Glx), creatine + phosphocreatine (total creatine, tCr), and lactate (Lac) etc. However, detection of GABA using the conventional ^{1}H-MRS is limited due to its relatively low concentration and the spectral overlap of signals from other major metabolites. An advanced MRS method, MEscherGArwood Point RESolved Spectroscopy (MEGA-PRESS) (Mescher et al., 1998; Puts and Edden, 2012) may be used to detect GABA levels in the healthy brain and compare them to levels in various pain and emotional disorders (Gao et al., 2013; Plante et al., 2012; Zunhammer et al., 2016). ^{1}H-MRS studies have revealed altered levels of cerebral neurotransmitters in various chronic pain conditions, such as fibromyalgia (Foerster et al., 2012), chronic back pain (Sharma et al., 2011; Zhao et al., 2017), and neuropathic pain (Widerstrom-Noga et al., 2013). Recently, we reviewed (Lv et al., 2017) the brain changes of CD detected by fMRI and MRS, and observed CD spectrum analysis of metabolites were commonly used in vitro, serum, urine, excreta and tissue samples. However, there have no studies to investigate the brain metabolites in vivo by using MRS in CD patients suffering from abdominal pain.

Based on previous studies, we hypothesized that CD patients with abdominal pain show not only changes in brain structure and function, but also altered neural metabolites levels in ACC. It additionally creates an abnormal rest activity brain region of CD patients with pain, which plays an important role in the perception, formation and regulation of pain. This study is aimed at determining alternations in metabolite levels, especially the neurotransmitters involved with pain, such as Glu and GABA, within the bilateral ACC of CD patients with abdominal pain using ^{1}H-MRS, and determining the relationship between metabolite levels and the clinical scores to further explore the neural mechanism in pain processing.

2. Methods

The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Zhejiang Chinese Medical University. All participants provided written informed consent.

2.1. Participants and study design

Sixteen CD patients with abdominal pain and 13 CD patients without abdominal pain confirmed by endoscopy or pathology, and were recruited from the First Affiliated Hospital of Zhejiang Chinese Medical University. The pain CD group included CD patients who experienced pain in the past 3 months, with the days of pain per week ≥ 3 days. The non-pain CD group included patients with CD who had not experienced abdominal pain in the past 3 months. Patients suffering from abdominal pain stemming from intestinal diseases other than CD, brain parenchymal lesions and mental or psychological disorders, recent (6 months) use of the CNS drugs, anodyne or recent (6 months) use of antidepressants or opioids, adverse life events resulting in temporarily depressed mood, and patients with claustrophobia or metal implants were excluded. Twenty healthy controls (HCs) were recruited with advertisements from Zhejiang Chinese Medical University. None of the HC subjects were taking any medication, or complained of gastrointestinal pain or pain related diseases. The clinical scores including hospital anxiety and depression scale (HADS), 0–10 Visual Analogue Scale (VAS) of pain and Crohn's disease activity index (CDAI) were evaluated before MR scan. According to the score of VAS, the CD patients divided into pain and non-pain CD groups. In the entire study, only 1 patient was excluded due to noise intolerance during scanning.

2.2. MR image and spectrum acquisition

Imaging of this study was conducted on a small aperture 3.0-Tesla MR scanner (Discovery 750, GE Healthcare, Milwaukee, WI),equipped with an 8-channel head phased array coil. Head motion was restricted by placement of comfortable paddings around the participant's head. A set of high-resolution T1-weighted structural images were obtained using three-dimensional BRAVO (brain volume imaging) sequence: TR = 8.2 ms, TE = 3.2 ms, FA = 12 degrees, matrix = 256 × 256, slice thickness: 1.0 mm with no gaps, was then used for the orientation and positioning of the subsequent ^{1}H-MRS scans. Point resolved spectroscopy (PRESS) was used to obtain single voxel spectrum. The voxel position for the single voxel spectroscopy in bilateral ACC (Fig. 1): TR = 2000 ms, TE = 35 ms, Voxel size = 20 × 20 × 20 mm^3, total number of scans = 64, number of excitations (NEX) = 8, water suppressed, with automatic shimming. Pre-scan requirements were < 8 Hz in automatic shimming at full width at half maximum (FWHM) and larger than 95% in water suppression. After the sequence of MEGA-PRESS was ready by GE Healthcare; nine CD patients with abdominal pain, 2 CD patients without abdominal pain and 8 HCs of those subjects were analyzed with MEGA-PRESS in the bilateral ACC to acquire GABA data: TR = 2000 ms TE = 68 ms, Voxel size = 20 × 20 × 20 mm^3 total number of scans = 64, NEX = 8, water suppressed. The voxel was carefully positioned within the ACC and outer-volume suppression (OVS) pulses around the ACC were facilitated for pre-saturation the lipid signals. Pre-scan requirements were same as PRESS acquisition.

2.3. MRS data processing

The raw P files of MRS were exported to a workstation equipped with linear combination model (LCModel) (Provencher, 1993) and Gannet (GABA analysis tool) (Edden et al., 2014) for processing (Fig. 2). This obtained the relative concentration (/tCr) and absolute concentration (mmol/L) of each metabolite, including Glu, Gln, GABA+, NAA, tCr, mIns etc. Only metabolites/neurochemicals processed by LCModel with Cramer-Rao Lower Bounds < 20% were analyzed. Since the resonances of Glu and Gln are overlapped quite a bit at 3T spectroscopy, they were labeled as Glx. However, Glu was also analyzed independently because it was determined reliably in high quality control of spectra at 3 T with short TE (Barker and Lin, 2006; Provencher, 1993). This study evaluated the relative concentration of metabolites (the relative ratio of tCr), due as tCr values were used as reference