In Vitro Hepatocyte Differentiation of Mesenchymal Stem Cells Derived from Human Bone Marrow

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Introduction: In addition to long-term self-renewal capacity, human mesenchymal stem cells (MSCs) possess versatile differentiation potential ranging from mesenchyme-related multipotency to neuroectodermal and endodermal competency. Of particular concern is hepaticogenic potential that can be utilized for liver-directed cell therapy and transplantation. Thus, this study examined whether human bone marrow-derived MSCs are able to differentiate into hepatocytes. Materials and methods: MSCs were cultured 2-step protocol with use of hepatocyte growth factor and oncostatin M. In the course of cell differentiation, cell morphology was observed by LM, EM and PLD activity and the expression of ALB, AFP, CK-18, CK-19, CPS, GS and GAPDH of hepatocyte were confirmed by Western blot analysis and RT-PCR. Hepatocyte functional activity were confirmed by glycerogen storage and uptake of low-density lipoprotein (LDL). Results: After 3 weeks of induction, cuboidal morphology, which is characteristic of hepatocytes, was observed, and also expressed marker protein specific of liver cell such as albumin, CK-18, and PEPCK. The presence of stored glycogen, as determined by PAS staining, was visualized at 4 weeks differentiation. After 6 weeks of differentiation, hepatocytes demonstrated the ability to uptake significant levels of LDL. Early phase of differentiation, we observed morphologic change and cell organelles including the Golgi body, mitochondria and ER by EM. Also PLD activity in the hepatic differentiation cells increased twofold or more at the 30 min point. Conclusions: MSCs from human bone marrow could differentiate into hepatocyte or hepatocyte-like cells in the differentiation media including HGF, FGF, EGF and OSM. Based on these observation, we conclude that human MSCs retain hepatogenic potential suitable for cell therapy and transplantation against intractable liver diseases.

S1804

Altered Kynurenine Pathway Metabolism in Irritable Bowel Syndrome (IBS) - Evidence of Indole-3-Glyoxylic Acid in Active Inflammatory Bowel Disease

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Background: IBS is a common disorder of the gastrointestinal tract (GIT) which affects 10-15% of the population. Although the pathophysiology remains unclear, it is increasingly being viewed as a disorder of the brain-gut axis. In particular, accumulating evidence points to the involvement of both the central and peripheral neurotransmitter systems in disease symptomatology. Serotonin, which is involved in regulating GIT secretion, motility and perception and its central role in the regulation of mood are also well documented. Low level immune activation has also been implicated in the IBS. The kynurenine pathway of tryptophan degradation has also been implicated in the IBS and the kynurenine pathway of tryptophan metabolism is considered to be a marker of immune activation, was measured using a commercially available ELISA assay. Results: Serum kynurenine levels (568.6 ± 60.1 vs 530.7 ± 24.3 ng/ml, p<0.05) and the kynurenine kynurenine ratio (0.005 ± 0.004 vs 0.049 ± 0.001 < p<0.01) were significantly increased in our IBS cohort. Neopterin levels (8.1 ± 1.8 vs 4.25 ± 0.29 mmol/L, p < 0.001) were also increased in the IBS subjects and the levels of the neuroprotective agent kynurenine (AGA) were decreased (3.23 ± 0.33 vs 6.75 ± 0.557 ng/ml) as was the kynurenine acid kynurenine ratio (0.0059 ± 0.0006 vs 0.0126 ± 0.0007 < p<0.01) Summary: These findings suggest that the activity of indoleamine-2,3-dioxygenase (IDO), the immunoresponsive enzyme which is responsible for the degradation of tryptophan along this pathway in a male IBS cohort.

S1805

Dietary Lectins As the Environmental “Unknown Pathogen” in Idiopathic Parkinson’s Disease

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Introduction: The pathogenesis of Parkinson’s disease (PD) remains unknown. Braak’s group has hypothesized that an “unknown pathogen” penetrates the gastrointestinal (GI) wall and enters the CNS via retrograde transport through the vagus nerve. This “unknown pathogen” induces glial cells and may cause a host response to glial cell injury, leading to a complex illness where they induce α-SYN aggregation. Using a rat model we have observed that incubation with lectin (300 nM), wild-type α-SYN (10µM) and Bodipy Maleimide conjugated α-SYN (10µM) induces α-SYN aggregation which was avoid when lectin was omitted from thesolution. FITC labeled cells are present in the brainstem of animals fed with 0.1% FTTC- lectin (300µl per onlay x3), whereas rats fed with vehicle or fluorescer tracer Diet did not show show any FITC signal in the CNS, and whole cell patchclamp recordings in thin brainstem slice showed that neuromelanin (100µM) excites a subgroup of gastric-projecting DMV neurons, indicating that DMV neurons express the lectin-like receptor and possibly the inclusion of LB. Dietary phytochemical lectins are indeed transfered and transported retrogradely from the GI tract to the CNS. Lectins, by virtue of their permeability, could also act as a chaperon for viruses and toxins, including those that may be responsible for α-SN inclusions in PD. It must be kept in mind, though, that the lectin-mediatated insult (if any) is gradual and might be determined by the association of lectins with other food structures.

S1806

Central Neurochemical Mechanisms of GES and Effects of Stimulation Parameters and Locations in Regular and Diet-Induced Obese Rats

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Gastric electrical stimulation has been used for the treatment of obesity with unclear mechanisms. The purposes of this study were to compare the difference in neuronal responses of the ventromedial hypothalamic VMH to GES between regular rats and diet-induced obese (DIO) rats and to optimize stimulation parameters and locations. Methods: Spontaneous unit discharges of 83 neurons in the VMH were recorded from 22 rats: 10 regular rats and 12 DIO rats. Gastric distention (GD) with 20 and 40 or 80mmHg was performed to determine whether the stronger stimulus may be more effective. The GD=0mHg was identified as a GD-responsive neuron, GES with different stimulation parameters (10, 20, 40 and 100Hz, pulse widths (0.1, 0.3, 0.6, 1.2 and 3ms), train-on times (0.1, 0.3, 1 and 2s) and amplitude (3, 6 and 10mA) was applied. VMH neuronal responses to GES were compared between regular and DIO rats. The percentage of responses to GES of 0.3ms in the DIO rats was significantly lower compared with that in the regular rats (P<0.03). 2) Different stimulation parameters resulted in different neuronal responses. For the stimulation frequency, there was an increase in neuronal responses from 10Hz to 40Hz (10Hz vs. 40Hz, p<0.001). However, GES of 100Hz did not yield a higher response rate than GES of 40Hz. For the pulse width, the neuronal response rate was proportional to the pulse width up to 3ms (0.1ms/3ms, p<0.001, 0.3ms/3ms, p=0.033; 0.6ms/3ms, p=0.020). Similar findings were noted with the train-on time (0.1s/2s, p<0.001, 0.5s/2s, p=0.045). For pulse amplitude, 6mA seemed to be sufficient (3mA/6mA, p=0.029; 6mA/10mA, p=0.889). GES of these parameters activated a vast majority (84%) of the tested neurons. 3) GES at distal antrum activated a higher percentage of neurons than GES at other locations (A/GC, p=0.008). Conclusion: DIO rats are significantly less responsive to gastrointestinal distention as well as gastric electrical stimulation. Based on the neuronal responses in the VMH, the best stimulation parameters for GES that dietary lectins transfered to the brain are 10Hz, 0.1ms pulse frequency of 40Hz, width of 3ms (or higher) and amplitude of 6mA. The best stimulation location seems to be the distal antrum. (The work was supported by Medtronic Inc.)

S1807

Cognitive Coping Mechanism of Limbic/Paralimbic System in the Modulation of Placebo Analgesia in Visceral Pain: A 3-T MRI Study

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Background Visceral pain is the cardinal symptom of functional GI disorder (FGID). Visceral sensation is also described as more unpleasant than somatic sensation, suggestive of higher cognitive/afffective components in visceral pain. Current treatment for FGID is often unsatisfying and associated with high placebo response. The central mechanism responsible for the placebo effect in visceral pain is unclear. Aim: To test the hypothesis that the placebo response to visceral pain is associated with the cognitive/afffective regions in brain. Method: 13 right-handed IBS cohort. Methods: We studied 101 healthy subjects (9F; 24.1±3.9 yrs) received brain fMRI in evaluating visceral pain evoked by ‘moderate’ painful esophageal balloon distention (22.7 ± 4.7 mmHg). Each subject received the same amount of saline would be given as a control. Pain catastrophization scale (PCS) was obtained from every subject before the task. Visual analogue scale (VAS) and short form McGill pain questionnaire were enquired after each session. Regression analysis to directly establish the relationship with intensity of placebo effect (r=-0.609, P=0.04), which suggests the thoughts and perception of placebo effect in visceral pain is associated with the cognitive/affective regions in brain. Results: 1) Pain intensity showed a linear relationship with intensity of placebo effect (r=-0.609, P=0.04). 2) PCS scores showed an inverse relationship with intensity of placebo effect (r=0.609, P=0.04). 3) The best neuronal response rate was proportional to the pulse width up to 3ms (0.1ms/3ms, p<0.001, 0.3ms/3ms, p=0.033, 0.6ms/3ms, p=0.020). Similar findings were noted with the train-on time (0.1s/2s, p<0.001, 0.5s/2s, p=0.045). For pulse amplitude, 6mA seemed to be sufficient (3mA/6mA, p=0.029; 6mA/10mA, p=0.889). GES of these parameters activated a vast majority (84%) of the tested neurons. 3) GES at distal antrum activated a higher percentage of neurons than GES at other locations (A/GC, p<0.008). Conclusion: DIO rats are significantly less responsive to gastrointestinal distention as well as gastric electrical stimulation. Based on the neuronal responses in the VMH, the best stimulation parameters for GES that dietary lectins transfered to the brain are 10Hz, 0.1ms pulse frequency of 40Hz, width of 3ms (or higher) and amplitude of 6mA. The best stimulation location seems to be the distal antrum. (The work was supported by Medtronic Inc.)

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(BA24), prefrontal (BA9,10,46), and superior temporal gyrus (BA22) were the regions positively correlated to the placebo effect measured by VAS or McGill pain rating index. **Conclusions:** (1) Significant reduction in pain and brain activation within pain related regions occurred during the placebo analgesia in visceral pain. (2) The correlation between the activation differences in limbic/paralimbic region and placebo intensity may suggest a cognitive coping mechanism in modulating placebo analgesia.

**S1808**

Exploring the Neural Processing of Visceral Sensations: the Influence of Sensory, Emotional and Cognitive Factors

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**INTRODUCTION:** Aetiology of Functional Gastrointestinal Disorders may be a result of aberrant processing of visceral sensations due to psychological disturbances.

A series of studies, we have used functional magnetic resonance imaging (fMRI) to assess the influence of psychological factors on the brain processing of oesophageal pain in healthy volunteers. **METHODS:** Study 1) 7 healthy volunteers (6 male, age range 20-25 years) underwent four separate fMRI scans incorporating four levels of phasic oesophageal stimulation (OS) ranging from non-painful to painful. Study 2) Whilst performing a distraction task, 12 healthy volunteers (all male, age range 21-32 years) underwent four separate fMRI scans incorporating four levels of phasic oesophageal stimulation (OS) ranging from non-painful to painful. In addition, a fifth scan was performed during painful OS without distraction. Study 3) 12 healthy volunteers (all male, age range 21-32 years) MRI images were acquired during two experimental runs in which volunteers received phasic painful OS under negative and neutral mood induction. RESULTS: Study 1) Mean VAS scores increased progressively with increasing OS intensity (r2=0.9, df 3, F=0.01). Bilateral activity in the anterior cingulate cortex (ACC) and primary somatosensory cortex (SI) was correlated with level and perceived intensity of OS (p<0.05, Bonferroni corrected). Study 2) Bilateral SI and left ACC activity increased with level of OS. Focusing attention on pain increased pain ratings and intensity of OS (r=0.3, df 0.05, corrected for multiple comparisons). Study 3) During negative mood induction, OS was associated with an increase (compared to neutral mood) in brain activity in the right anterior insula and right ACC (p<0.05, corrected for multiple comparisons). CONCLUSIONS: Bilateral SI and left ACC appear to encode sensory aspects of OS such as intensity. Activity in the right ACC is altered by attention, and both right insula and right ACC are modulated by negative mood induction suggesting involvement of these regions in cognitive and emotional components of pain processing. Evidence of right hemispheric dominance during emotional and cognitive manipulation suggests the roles the right hemisphere plays in the modulation of visceral sensitivity (arousal, negative affect) and that the right insula and right ACC are integral for subjective awareness of emotion. These studies provide new insights into complex interactions that comprise the visceral pain neuraxis which may be important in future experiments applied to studying functional clinical disorders that have pain as a primary symptom.

**S1810**

Evaluation of Spino-Anorectal Pathways in Spinal Cord Injury with Bowel Dysfunction Using Magnetic Stimulation: A Novel and Noninvasive Test

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**INTRODUCTION:** Spinal cord and cauda equina injury can cause distressing symptoms and affect bowel function. Currently, there is no specific test for evaluating these problems as well as spino-anorectal pathes. Pudendal nerve latency (PNTML) provides limited and conflicting data. AIM: We tested the hypothesis that motor evoked potentials (MEP) of anorectum using translumbar and transsacral magnetic stimulation provides comprehensive pathophysiologic information regarding spino-anorectal pathways in patients with spinal cord injury when compared to PNTML. METHODS: 22 (M/F 9/13) patients with history (median duration=3 yrs) of spinal cord injury (cervical-2, thoracic-2, lumbosacral-14, multilevel-4), mean age 49±16 yrs and 11 healthy subjects were assessed. Magnetic stimulations were performed with a Cadwell Farapoint Coild™ (9-cm) placed over the right and left of midline at L3-L4 and SI-S3 levels using 80%-100% intensity. Anal and rectal MEPs were measured after lumbar and sacral stimulations by placing a novel probe containing 2 pairs of bipolar steel ring electrodes, located in the rectum and anal canal. PNTML (St. Mark’s electrode) and MEP latencies were compared and their clinical utility was assessed. RESULTS: MEPs were significantly prolongned on both sides and in the lumbar and sacral regions in patients compared to controls (Table). Abnormal MEPs were detected in 16 (80%), 13 (65%), 14 (70%), and 11 (55%) patients respectively in the lumbo-anal (TL-aMEP), lumbo-rectal (TL-rMEP), sacro-anal (TS-aMEP), and sacro-rectal (TS-rMEP) tracts. A single abnormal MEP was identified in 20 (100%) patients and abnormal PNTML in 13 (65%) patients (p<0.05). Abnormal MEP was detected on right side in 19 (95%) and left side in 20(100%) and abnormal PNTML in 13(65%) patients on each side. MEPs and PNTML were unobtainable in 2 patients each from technical problems. CONCLUSIONS: Translumbar and transsacral MEPs provide clear delineation of the spino-anorectal and spino-rectal pathways and reveal significant neuropathy in spinal cord injury patients. It is a superior test to PNTML. The test is well tolerated and offers a safe, inexpensive, and objective method of evaluating peripheral brain-gut pathways and hitherto unknown information regarding pelvic floor neuropathy and enhance diagnostic yield.

**S1811**

The Neurophysiology of Gastric Sensation in Functional Dyspepsia: Role of Abuse History and Somatization

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Background Abuse history and somatization are prevalent in Functional Dyspepsia (FD), but the exact nature of the relationship between both is incompletely understood. Abuse history influences neural correlates of rectal distension in Irritable Bowel Syndrome (Ringel et al., DDW 2006), but this has not been studied in FD, neither has the influence of somatization. AIM: To study the influence of abuse history & somatization on neural correlates of gastric distension in FD. Methods Brain H2(1)O-PET was performed in 25 FD patients (mean age 33) during 3 conditions (random order): no distension (baseline), isobaric distension at 15 cmH2O & sham distension. Somatization (PHQ-15) & abuse history were measured using validated self-report questionnaires. Data were analyzed with threshold p<0.001 (SPM2). Abuse history & somatization were correlated with mean brain activation during baseline, distension and sham as well as with the mean differences in activation distension-baseline and sham-baseline. Results Behavioral Mean score for somatization was 15±1, with 15 being the cutoff for high (November 2002). Eight patients (32%) reported an overall history of abuse; this was associated with significantly higher somatization scores (19±2 v.s 13±2, p<0.01). Imaging results are summarized in the table. Significant correlations were found between abuse history and activation in mainly (para)limbic areas involved in affective regulation including hippocampus, amygdala, orbitofrontal cortex and insula. Somatization mainly correlated with brainstem (medulla), cerebellum, cingulate subregions and higher-order association areas. Conclusion In FD, brain activation during baseline, gastric distension and sham is influenced by abuse history and somatization. These findings provide neurobiological correlates for psychosocial influences on gastric sensation and their involvement in FD pathogenesis.