

New Phenomenon

Homocysteine-mediated intestinal epithelial barrier dysfunction in the rat model of irritable bowel syndrome caused by maternal separation

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Irritable bowel syndrome (IBS), a common functional gastrointestinal disorder, is characterized by altered bowel evacuation, bloating, and visceral pain in the absence of anatomical or biochemical abnormalities [1]. Up to date, the etiologies of these symptoms are still not fully understood. The maternal separation (MS) model, rodent pups separated from the dam (3 h/day) during the neonatal period, has been found to display greater anxiety-like behavior, visceral hypersensitivity, increased fecal output, and higher levels of proinflammatory cytokines in adulthood [2], which may constitute a valuable experimental model to investigate the pathophysiology of IBS and to identify novel pharmacological targets. The rat model of IBS caused by MS was established in this study. All of the investigations conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

Homocysteine (Hcy), a cytotoxic sulfur-containing amino acid, is an intermediary metabolite in methionine/cysteine metabolism. The elevated plasma level of Hcy is termed as hyperhomocysteinemia (HHcy). And the regulation of Hcy is critically important for health maintenance since several diseases are related to HHcy [3,4]. Augmentations in Hcy are due to enzyme abnormalities in metabolic pathways or to nutritional deficits, including folate and Vitamin B₆ and B₁₂ deficiencies [5]. The gastrointestinal tract is a significant site of sulfur amino acid metabolism, which accounts for 25% of whole body transmethylation and transsulfuration pathways and is a prominent site of net Hcy release [6]. The demonstration of a relationship among Hcy, inflammation, and autoimmunity intriguingly expands the spectrum of the possible pathogenetic implications for HHcy. However, there is a paucity of studies investigating its role in the context of IBS.

In our study, we found that plasma Hcy levels in IBS rats were significantly greater than that of control (7.26 ± 1.84 vs. 3.6 ± 1.0 $\mu\text{mol/l}$, $P < 0.01$, **Fig. 1A**), which was increased ~ 1 -fold. After administration of folate, Vitamins B₆ and B₁₂, Hcy levels were remarkably decreased (5.02 ± 1.48 $\mu\text{mol/l}$, $P < 0.05$), which reduced $\sim 30\%$ of the IBS. To our knowledge, this was the first report showing that MS

stress could lead to Hcy accumulation in the circulation, and there was a significant association between Hcy levels and the severity of IBS symptoms including visceral hyperalgesia and stress-induced increase in colonic motility. Thus, Hcy may be implicated in the onset and progression of the tissue injury underlying IBS.

Recently, researchers have found that a low-grade colonic inflammation may play a role in the pathogenesis of IBS [7]. Disruption of the intestinal epithelial barrier is a feature of gut inflammation in humans and is a pathogenic factor in IBS [8,9]. Previous studies have shown that MS predisposed adult rats to colonic barrier dysfunction in response to mild stress as a stress factor owing to the exaggerated expression of cytokines [10]. Our results showed that plasma TNF- α levels in IBS rats were significantly higher than those in control (443.6 ± 8.18 vs. 383.0 ± 11.13 pg/ml , $P < 0.01$, **Fig. 1B**). After treatment with Hcy-lowering reagent, the level of plasma TNF- α was markedly declined (411.0 ± 7.8 pg/ml , $P < 0.05$), but still higher than that in the control. These data indicated that Hcy probably acted as a proinflammatory molecule triggering inflammatory effects in IBS course.

Furthermore, intestinal permeability was assessed using a sugar probes test including sucralose, lactulose, and mannitol by HPLC-tandem mass spectrometry analysis in this study. Lactulose and sucralose permeate the intestinal epithelial barrier through paracellular junctions, and mannitol is a marker of transcellular permeation. There is no difference for urine mannitol excretion in 0–5 h collection following administration of sugar probes among three groups (control: 1601 ± 48.85 μg ; IBS: 1598 ± 122.3 μg ; IBS + Vit B: 1541 ± 155.3 μg , **Fig. 1C**), while the ratio of lactulose to mannitol in 0–5 h collection was significantly increased by 47% in IBS rats compared with that in control (0.22 ± 0.07 vs. 0.15 ± 0.03 , $P < 0.01$, **Fig. 1D**). After Hcy-lowering treatment, the ratio of lactulose to mannitol was markedly declined (0.17 ± 0.03 , $P < 0.05$). These results suggested that paracellular permeability of small intestine was increased based on urine mannitol and lactulose excretion analysis. Meanwhile, urine sucralose excretion in 6–24 h collection

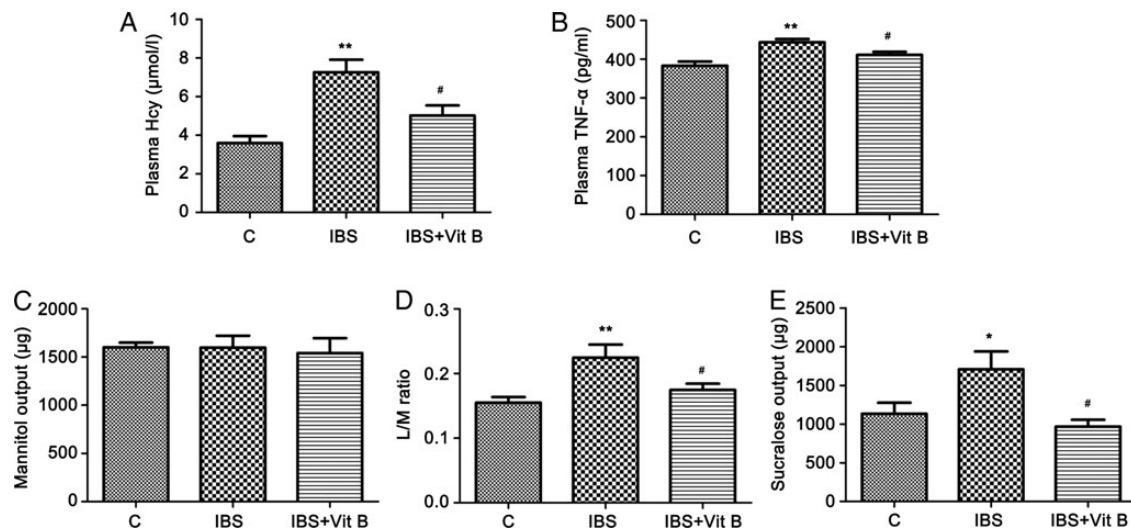


Figure 1. Effects of Hcy on intestinal barrier function and plasma TNF- α levels in IBS rat caused by maternal separation (A) Plasma Hcy levels detected by HPLC assay. (B) Plasma TNF- α levels detected by ELISA. (C) Mannitol output in 0–5 h urine collection after administration of three sugar probes detected by HPLC–MS/MS analysis. (D) Lactulose/mannitol (L/M) ratio in 0–5 h urine collection. (E) Sucralose output in 6–24 h urine collection. Data are expressed as mean \pm SD ($n = 8$). One-way ANOVA with Tukey's *post hoc* test was performed. * $P < 0.05$, ** $P < 0.01$ vs. C; # $P < 0.05$ vs. IBS. C, control; IBS, irritable bowel syndrome; IBS + Vit B, irritable bowel syndrome with administration of vitamin B.

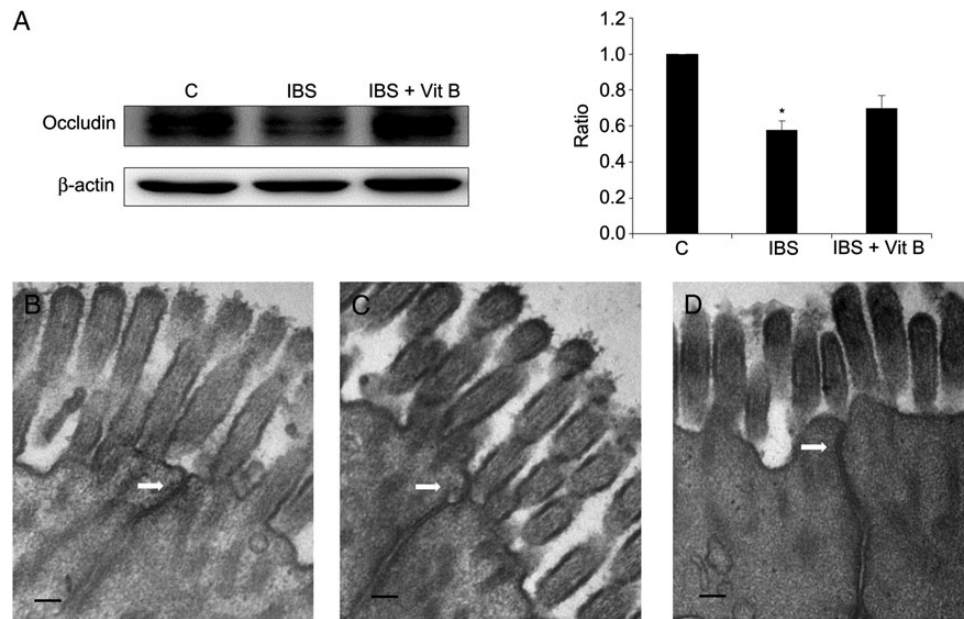


Figure 2. Excess Hcy disrupt the integrity of TJs in the colon of IBS rat (A) Inhibition of occludin expression in IBS rats detected by western blot analysis. β -Actin was used as an internal control. The change ratio of protein is normalized by β -actin based on the OD value of protein bands, and graphical representations of the protein are shown in the right panel. (B) In control, TJs of cell membranes of adjacent cells are in close apposition revealed by transmission electron microscopy. (C) Typical dilatation of TJs was observed in IBS rats. (D) This injury effect was mitigated by Hcy-lowering treatment. Arrows show TJs. Scale bar, 100 nm. ($n = 3$). * $P < 0.05$ vs. C. C, control; IBS, irritable bowel syndrome; IBS + Vit B, irritable bowel syndrome with administration of vitamin B.

which reflects colonic paracellular permeability in IBS rats was higher than that in control (1707 ± 230.9 vs. $1135 \pm 140.9 \mu\text{g}$, $P < 0.05$, **Fig. 1E**), Hcy-lowering treatment affected intestinal permeability ($967.1 \pm 88.56 \mu\text{g}$, $P < 0.05$) and could restore barrier function. Urinary volumes were similar in all groups in 24 h. The data presented herein

support that excess levels of Hcy may be deleterious to intestinal epithelial barrier.

The structural integrity of the intestinal epithelium is maintained by three distinct adhesion systems: tight junctions (TJs), adherent junctions, and desmosomes. Of these, TJs comprise the most apical component and are the rate-limiting

step for paracellular permeability [11]. To understand the mechanism by which Hcy affects intestinal paracellular permeability, TJ transmembrane protein occludin was analyzed by western blot analysis. Compared with control, the total protein in the colon of IBS rats was considerably decreased by ~42% ($P < 0.05$, **Fig. 2A**). Besides, Hcy-lowering treatment prevented the loss of occludin, which restored to 70% of the control. Moreover, the ultra-structural damage of TJs was detected in IBS colon by transmission electron microscopy (**Fig. 2B-D**). The results suggested that elevated levels of Hcy could down-regulate occludin expression and change TJs morphology, thus resulting in an increased paracellular permeability, while there is a benefit of Hcy-lowering treatment in preserving TJs integrity.

In conclusion, we reported the novel finding that the existence of increased Hcy at circulating levels in a MS-induced IBS rat may contribute to the intestinal epithelial barrier defects associated with IBS symptoms such as sensorimotor abnormality. The data demonstrated that a persistent increase in Hcy was related to breakdown of TJs and increased intestinal paracellular permeability in IBS, in which the damaging effect could be alleviated by folate, Vitamin B₆ and B₁₂ supplementation, a clinical therapeutic strategy for HHcy, and it may be beneficial in IBS treatment. However, whether the increased plasma Hcy levels in IBS rats is the cause of the intestinal disorder or a consequence of an existing inflammatory response needs to be further studied.

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