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## HPLC ENANTIOMETRIC RESOLUTION OF ONDANSETRON ENANTIOMERS ON CHIRAL STATIONARY PHASES

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**Background:** Ondansetron, 1,2,3,9-tetrahydro-9-methyl-3-[(2-methyl-1H-imidazol-1-yl) methyl] -4H-car-bazol-4-one, is a highly selective and potent 5-HT3 receptor antagonist with antiemetic action. It exhibits little or no sedation or extrapyramidal side effects associated with other commonly used antiemetic drugs such as metoclopramide, prochorperazine or droperidol.

**Methods:** New chromatographic conditions were used to separate ondansetron enantiomers on three polysaccharide stationary phase. Stock solutions (1 mg/mL) were prepared by dissolving 10 mg of racemic ondansetron in methanol in a 10 mL volumetric flask. The solutions were stored at 20°C. The solutions were filtered through a 0.45 μm syringe type filter and sonicated 15 minutes before use. To improve sensitivity, mobile phase pH, the percentage of organic modifier, and the flow rate were optimized. The effect of organic modifiers on resolution of ondansetron enantiomers has been studied at room temperature under similar mobile phase conditions.

**Results:** Enantiomeric purity of ondansetron was determined by HPLC on a Chiralpak AS-H chiral column. The mobile phases were used by hexane - ethanol-acetonitrile (80:19:1, v/v). The flow rate was 1 mL/min. The UV detection wavelength was 302 nm. Under the same condition, the resolution capability of these chiral stationary phases were in the order Chiralpak AS-H ($R_s$ 2.87) > Chiralcel OD-H > Chiralpak AD-H. The resolution capability of these chiral stationary phases were in the order Chiralpak AS-H ($R_s$ 2.87) >Chiralcel OD-H > Chiralpak AD-. A different geometry and size of the helical groove, which may be influenced by the addition of polar organic modifiers to the mobile phase, is probably the main reason for different separation properties.

**Conclusions:** In summary, this article successfully established a method for separating ondansetron enantiomers on Chiralpak AS-H by HPLC. The effects of the percentage of organic modifiers, the flow rate and mobile phase pH on separation of enantiomers were investigated in detail.

**Acknowledgments:** We are grateful for the financial support of the National Natural Science Foundation of China (21462018), the Science Fund of the Technology Office of Jiangxi, China (20122BAB203017 and 20132BBE50024).

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## WUMEIWAN AMELIORATES TNBS-INDUCED COLITIS IN MICE BY AFFECTING $CD4^+CD25^+FOXP3^+$ REGULATORY T CELLS

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**Introduction:** Although the etiology of IBD remains elusive, significant research has been carried out that an increased influx of neutrophils and macrophages accompanied by the secretion of proinflammatory cytokines is considered to exacerbate IBD. Some studies show that $CD4^+CD25^+FoxP3^+$ regulatory T cells have been shown to ameliorate inflammatory colitis mainly by the production of IL-10 and TGF-β. So, Treg is confirmed as a new therapeutic target to treat severe inflammatory colitis. The famous prescription "Wumeiwan" (WMW) in "Shanghan Lun" was widely used for thousands of years to treat patients with colitis. However, there were no reports available about whether WMW effective mechanism is related to Treg. Therefore, the purpose of this study was to investigate the possible effect of WMW on Treg cells in TNBS-induced ulcerative colitis mice.

**Methods:** Ninety mice were randomly divided into normal group, normal control (CON) group, untreated control (TNBS) group, dexamethasone (DEX) group, Wumeiwan high (HWMW) group, Wumeiwan middle (MWMW) group, and Wumeiwan low (LWMW) group, except normal group,

Acute colitis was induced in mice by treatment with trinitrobenzene sulphonic acid (TNBS), mice in the control group and TNBS group were all orally given sterile saline once a day, and mice in the DEX group were given dexamethasone at 1, 3, 5, 7, 9 days after TNBS administration. Mice in the HWMW, MWMW and LWMW group were orally treated by gavage with WMW at different doses respectively. Ten days later, Treg cells of colonic lamina propria cells were detected by flow cytometry (FCM). Expression of Foxp3, IL-10, mRNA, and TNF-α, IL-1β, and IL-10 in colon was inspected by real-time PCR and ELISA respectively.

**Results:** The WMW significantly attenuated TNBS-induced Macroscopic score. The WMW also effectively prevented shortening of colon length and crypt length. Histological analysis indicated that WMW suppressed epithelial damage, loss of goblet cells, loss of crypts, and infiltration of inflammatory cells induced by TNBS. In addition, WMW inhibited TNF-a, IL-1β and IL-10 expression, decreased myeloperoxidase (MPO) in colon tissue more important, a significant increase in FoxP3 expression was found in isolated lamina propria CD4+ T cells, as well as FoxP3 and IL-10mRNA expression in WMW-treated mice.

**Conclusions:** The WMW shows significantly protective effects on mice with colitis induced by TNBS. The mechanism may be due to the improvement of Treg in colonic.

**Acknowledgments:** The National Natural Science Foundation of China, Foundation Number: 81173177; Henan University of Traditional Chinese Medicine Provincial Scientific Projects: Foundation Number: 2014KYYWF-QN10.

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## GENDER-SPECIFIC EFFECT OF DEFICIENT ABCG4 ON LIPOGENESIS IN A MOUSE BRAIN

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**Introduction:** Abcg4 is a member of the G subfamily of ATP-binding cassette transporters (ABC) which uses ATP hydrolysis to transport a wide variety of substrates across various cellular membranes. Abcg4 is highly expressed in the brain and neural retina of the eyes as assessed by northern blot analysis. Abcg1 and Abcg4 have been shown to mediate cholesterol efflux to HDL in the brain. Abcg4 may play an important role in the development of Alzheimer disease, since Abcg4 was found highly expressed in the microglia near the senile plaque in the brain of a patient with Alzheimer disease. Bojanic DD et al has recently examined Abcg1 and Abcg4 expression and function during development and aging. They found that loss of both Abcg1 and Abcg4 results in accumulation in the retina and/or brain of oxysterols, and behavioral tests showed that Abcg4−/− mice had a general deficit in associative fear memory. The data indicated that the Abcg4 plays a critical function in the central nervous system (CNS).

Sex-specific effect on the gene expression has been reported in different organisms. Another member of ABCG transporters, Abcg2 or BCRP (breast cancer resistance protein), has also been found to have sex-dependent expression and activity in the liver. The relationship of Abcg4 expression and lipogenesis with regard to gender difference in a mouse's brain as well as in the body remains much to be explored.

**Methods:** Gene-knockout mouse: Abcg4 knock-out/GFP knock-in mice were made and genotypically verified with southern blot. Sex-matched littermates were chosen for the study. Quantitative PCRs were performed with SYBR-Green PCR Master Mix of Applied Biosystems using the 7300 Real-time PCR machine. The real-time PCR results were automatically analyzed and produced by ABI 7300 SDS software and were saved in the "print screen" format or in an exported raw-data form.

**Results:** HMGS, FAS and HMGR, SREBP2, and SR-B1 mRNAs were significantly upregulated in the brains of Abcg4 knockout female mice, 28%, 25%, 22%, 20%, and 17% respectively higher than that of wild type female mice. But in male mice brains, mRNA expressions for other genes, such as LXRa, ApoE, Abca1, and LDL-R, were significantly up regulated in the knockout mice, and were 83%, 44%, 36%, and 27% higher than that of their wild type mice respectively. SREBP1c were also remarkably (31% higher of that of wild type) upregulated in the brain.