DOCTORAL THESIS

Modulatory effect of magnolol in colonic motility dysfunction induced by neonatal maternal separation in rats

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Modulatory Effect of Magnolol in Colonic Motility Dysfunction Induced by Neonatal Maternal Separation in Rats

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ABSTRACT

Colonic motility dysfunction is one of the main clinical features of irritable bowel syndrome (IBS), which is a common disease of gastrointestinal functional disorders. Stress plays an important role in the onset and development of IBS and can cause the colonic motility dysfunction. The etiology colonic motility dysfunction has not been clarified and current therapies are not perfect. Magnolol, 5,5′-Diallyl-2,2′-biphenyldiol, is a bioactive compound found in the bark of the herb *Cortex Magnoliae Officinalis*, which has been used for lower gastrointestinal disorders such as diarrhea and constipation in Traditional Chinese medicine (TCM) for long time but the mechanisms have not been well understood. The aims of this study are: 1) to investigate the colonic motility status induced by neonatal maternal separation (NMS); 2) to investigate whether NMS-induced colonic motility dysfunction is related to the change of L-type calcium channels; 3) to investigate the pharmacological effect of magnolol on colonic motility function in NMS rats; and 4) to investigate the possible underlying mechanism of magnolol's effect.

In *in vivo* study, the changes of colonic transit were detected through water avoidance stress (WAS) test. In *in vitro* study, the spontaneous contraction and smooth muscle contractions of colonic smooth muscles under different ligands were recorded through an organ bath system. Laser confocal fluorescent imaging study method was used to compare intracellular calcium concentration ($[Ca^{2+}]_i$) under different agents in enzymatically isolated single colonic smooth muscle cells of rats. The change of L-type

Ca^{2+} channels currents in the single colonic smooth muscle cells were recorded by using patch clamp technique. Also the immunofluorescence and Western blotting analysis were used to compare the expression of relative proteins.

The results of this study are summarized as follows:

1. NMS increased (1) the fecal pellet number in response to one hour water avoidance stress; (2) the amplitude of spontaneous contraction of colonic sections; (3) contractile responses induced by L-type calcium channels activator Bay K 8644, high K^+ ions and ACh; and elevated contractile activities was accompanied with an increase of $[Ca^{2+}]$i in colonic myocytes; (4) the expression of L-type Ca^{2+} channels α_{1c}–subunit protein in colonic smooth muscle. These data indicate that NMS induces colonic motility disorder, which is associated with the up-regulation of L-type Ca^{2+} channels in colonic smooth muscles.

2. Magnolol dose-dependently inhibited (1) the colonic motility under one hour WAS *in vivo* in NMS rats; (2) the colonic smooth muscle spontaneous contraction and contractile effect induced by different stimuli *in vitro* in NMS rats; (3) the increased colonic motility pretreatment with Bay K 8644 under 1 hour WAS *in vivo* with nonhandled (NH) rats; (4) $[Ca^{2+}]_i$ of colonic smooth muscle cells in NMS rats. These data suggest that magnolol can regulate abnormal colonic motility, and the effect may be associated with the inhibitory effect on activity of voltage-sensitive L-type calcium channels,

3. Magnolol dose-dependently inhibited 1) the spontaneous contractions of colonic smooth muscle and Bay K 8644-, ACh- and KCl-induced contractions in the rat colons *in vitro* in NH rats; 2) L-type Ca^{2+}- channel currents in isolated single colonic smooth muscle cell; 3) expression of α_{1c}-subunit of L-type Ca^{2+} channels in rat colonic smooth muscles. These results suggest that magnolol caused the relaxation effect on distal colonic smooth muscle in rat through inhibition of L - type Ca^{2+} channels activities and expression in smooth muscle cells, indicating that the magnolol could act as an L-type calcium channels blocker, and has the potential to be used as an agent for treating IBS.

4. Magnolol at the concentration of 10 μM - 100 μM inhibited smooth muscle contractility in the presence of Ca^{2+} but had no inhibitory effect in the absence of Ca^{2+} after activation of PKC with 30 nM phorbol 12-myristate 13-acetate (PMA). Western blotting results showed that magnolol inhibited the expression of MYL9 (MLC_{20}) on smooth muscle but could not affect the expression of MLCK and CPI-17 on smooth muscle. These data suggest that the inhibitory effect of mangolol is involved in the Ca^{2+} dependent PKC pathway. Furthermore, the effect is accompanied by a reduction of MLC_{20} on the colonic smooth muscle.

In conclusion, the results highlight the role of L–type Ca^{2+} channels in the pathophysiological mechanism of NMS-induced colonic motility dysfunction, and clarify that the magnolol can inhibit the colonic muscle contraction; and this effect is mediated through the L-type Ca^{2+} channel signaling. These data indicate that magnolol

could be used as a potential L-type calcium channels blocker to treat IBS.

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