DOCTORAL THESIS

Toward novel therapeutics for functional constipation: from traditional Chinese medicine herbal formula MaZiRenWan to cyclic spexin analogues

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ABSTRACT

Functional constipation (FC) is a major gastrointestional (GI) disorder which affects about 14% population worldwide. However, due to efficacy and safety concerns, more than 50% FC patients are not completely satisfied with current conventional therapies, thus alternative therapies are needed. A substantial part of FC patients have symptom of slow colonic motility, while therapy targeting a single pathway cannot benefit all of them. In this thesis, we searched for novel FC therapeutics from two distinct sources, both of which can improve colonic motility significantly: (1) *MaZiRenWan* (MZRW), an herb formula from Traditional Chinese Medicine (TCM); and (2) Spexin (SPX), a newly identified neuropeptide that is deregulated in FC. On the basis of efficacy validation for MZRW by randomized, placebo-controlled clinical studies, we investigated the bioactive compounds and pharmacological actions of MZRW. Firstly, a machine-learning based method, namely MOST, was developed to relate bioactive compounds with their mechanism-of-action targets. MOST demonstrated good performance in 7-fold cross-validation (over 87% accuracy) and temporal validation (over 76% accuracy). In the case laxative effect, MOST predicted that acetylcholinesterase (ACHE) was the mechanism-of-action target of aloe-emodin; *in vivo* studies validated this prediction. Secondly, we analyzed the bioactive compounds and mechanism-of-actions of MZRW with combination of UPLC-QTOF-MS/MS, clustering analysis, organ bath, and MOST approaches. 97 compounds were identified in MZRW extract, and 35 of them can be found in plasma and feces samples of rats with oral

administration of MZRW. Chemical space analysis suggested that these compounds can be classified into component groups, while the corresponding pharmacology can be studied with representative compounds. Emodin, amygdalin, albiflorin, honokiol, and naringin were shown to induce spontaneous contractions of rat colonic smooth muscle *in vitro*. Biological targets in ACh-, estrogen-, prostaglandin-, cannabinoid-, and purine signaling pathways are able to explain the prokinetic effects of representative compounds and component groups. Pharmacological actions of MZRW are mixture of five classic paradigms. Thirdly, the latest results of three-armed, randomized and controlled clinical study showed that MZRW demonstrated comparable efficacy with the first line drug Senna, the first line drug for constipation in HK, during treatment period, both were better than placebo; and the efficacy was more sustainable in follow-up period when comparing that of Senna and placebo. These data suggested the unique pharmacological profile of MZRW for FC. With pharmacometabolomic analysis, we found that change of oleamide is negatively correlated (pearson r = -0.59, $p<0.001$) with improvement of Complete Spontaneous Bowel Movement (CSBM) in MZRW group, but not in Senna or placebo group. Oleamide is up-regulated in FC patients compared with healthy controls, and MZRW can significantly reduce oleamide in FC patients (n=30), healthy human volunteers (n=23), and in normal mice (n=12) serum, ileum, and colon. The regulation of oleamide by MZRW is possibly via augmenting FAAH-mediated degradation. Lastly, we investigated the possibility to use SPX, the newly identified, FC-associated neuropeptide to change

GI motility. The deregulation of SPX has been found in several disorders including FC, however, the metabolic instability of SPX prevent it to be directly used in clinical practices. Our investigation through combination of molecular dynamics (MD) simulations and NMR analysis suggested a β-turn-helix-β-turn (βαβ) conformation for human spexin (hSPX) adopts in solution. Consistent with this conformation, cyclic analogues of hSPX with a disulfide bond between residue 1 and 13, LH101 (CWTPQAMLYLKGCQ-NH_2), activated both GalR2 (EC_{50}=1.19 μM) and GalR3 (EC_{50}=1.56 μM) with potency comparable to wild type, and that the acetylation at the N-terminal, LH101(Ac) raises the potency EC_{50}=0.38 μM on GalR2 and EC_{50}=0.39 μM on GalR3. The serum half lives of LH101 ($t_{1/2}$=355.7 min) and LH101(Ac) ($t_{1/2}$=1973.7 min) were significantly longer than the wild type ($t_{1/2}$=66.5 min), and LH101(Ac) induces the contractions of mice intestinal segment *in vitro* and attenuates the oleamide-induced slow GI motility *in vivo*. Collectively, our studies in MZRW suggested that estrogen and oleamide signaling pathways are potential new targets to develop novel therapeutics for FC, while lead compounds targeting these pathways could be found from MZRW. The final study suggested CSAs have potential to be developed as new FC therapy by targeting the galanin receptor associated pathway.

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