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Extracts from peppermint leaves, lemon balm leaves and in particular angelica roots mimic the pro-secretory action of the herbal preparation STW 5 in the human intestine

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ABSTRACT

Aim: The herbal preparation STW 5 contains fresh plant extracts from bitter candytuft whole plant, extracts from greater celandine herb, angelica root, lemon balm leaves, peppermint leaves, caraway fruit, liquorice root, chamomile flower and milk thistle fruit. We recently reported that STW 5 increased intestinal chloride secretion and proposed that this action may be involved in its clinical efficacy in the treatment of irritable bowel syndrome. The aim of this study was to identify the extracts responsible for the secretory action in order to provide the basis to develop novel target oriented herbal combinations.
Methods: We used the Ussing chamber voltage clamp technique to study the effects of individual extracts of STW 5 on short circuit current (Isc, reflecting electrogenic ion transport across epithelial cells) in mucosal/submucosal preparations of human small or large intestinal specimens and the human epithelial cell line T84.
Results: STW 5 at concentrations of 512 μg/ml and 5120 μg/ml evoked an increase in Isc. The increase at the lower concentration was due to pro-secretory effects of angelica which were nerve mediated. The increase at the higher concentration was additionally mimicked by peppermint and lemon balm. The remaining extracts did not influence I_{SC} in the large intestine. The results were similar in T84 cells except that angelica had no effect while chamomile induced secretion. These pro-secretory effects were reduced by adenylate cyclase inhibitor MDL-12330A, cystic fibrosis transmembrane conductance regulator (CFTR) inhibitor $CFTR_{inh}$-172 and calcium activated chloride channels blocker 4-acetamido-4-isothiocyanatostilbene-2,2-disulphonic acid (SITS). Liquorice decreased I_{SC} only in small intestine which was reversed by the epithelial sodium channel blocker amiloride.
Conclusions: Results suggested that the pro-secretory action of STW 5 is mainly due to angelica with lesser contribution of peppermint and lemon balm. Their effects involve activation of cAMP- and Ca^{++}-activated Cl^- channels. We suggest that peppermint, lemon balm and in particular angelica may be the basis to develop novel herbal preparations to specifically treat secretory disorder based on impaired epithelial secretion, such as constipation.

Introduction

Irritable bowel syndrome is a functional bowel disorder characterized by stool irregularities. Some newly developed drugs, such as lubiprostone and linaclotide specifically target intestinal secretion in order to improve in particular constipation (Andresen et al. 2007; Johanson et al. 2008; Thomas and Allmond 2013).

Herbal medicine is increasingly used to treat in particular functional gastrointestinal diseases (Brierley and Kelber 2011; Rahimi and Abdollahi 2012). One of these medications is the herbal preparation STW 5, which is successfully used for over 50 years to treat

Abbreviations: APL, angelica + peppermint + liquorice; APLC, angelica + peppermint + liquorice + chamomile; cAMP, cyclic adenosine monophosphate; Ca-Cl, calcium dependent chloride channel; CFTR, cystic fibrosis transmembrane conductance regulator; DMSO, dimethyl sulphoxide; I_{SC}, short circuit current; SITS, 4-acetamido-4-isothiocyanatostilbene-2,2-disulphonic acid; TTX, terodotoxin.

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patients with functional dyspepsia (Madisch et al. 2001; Melzer et al. 2004; Madisch et al. 2004a). STW 5 and is a fixed herbal combination of a fresh plant extract from bitter candytuft (Iberis amara) and extracts from dried greater celandine herb, angelica root, lemon balm leaves, peppermint leaves, caraway fruit, liquorice root, chamomile flower and milk thistle fruit (Wegener and Wagner 2006). There is one clinical study suggesting that STW 5 was also beneficial in patients with irritable bowel syndrome (IBS) improving visceral pain as well as the stool irregularities (Madisch et al. 2004b). We reported recently that STW 5 increased ion secretion in the human small and large intestine and proposed that this pro-secretory activity may be involved in its clinical efficacy (Krueger et al. 2009). The increased secretion was mediated by opening of cAMP and calcium dependent chloride channels because the adenylate cyclase inhibitor MDL-12,330A as well as 4-acetamido-4-isothiocyanatostilbene-2,2-disulphonic acid (SITS) antagonized the actions of STW 5. In the previous study we did not address the question which of the individual components was responsible for the enhanced ion secretion. Results of such a study may further demonstrate the potential for selective disease-targeted combinations and particularly stimulate development of specific herbal preparations that target epithelial chloride secretion.

We therefore aimed to identify the plant extract or extracts that were responsible for the secretory action of STW 5 and studied its effect on human small and large intestinal preparations as well as in the T84 epithelial cell line.

Material and methods

All experimental procedures have been previously published in detail (Krueger et al. 2009, Krueger et al. 2013).

Tissue samples

Tissue preparations were obtained from 119 patients with a mean age of 64 ± 15 years (ranging from 14 to 93 years) who underwent routine surgical operations at the hospitals in Freising and Klinikum Rechts der Isar of the Technische Universität München. Diagnoses that led to surgeries were (number of patients in parenthesis): stomach carcinoma (15), pancreatic carcinoma (10), large intestine carcinoma (55), diverticulitis (7), ovarian carcinoma (3), polyps (3), allergic eosinophilic gastroenteritis (AEG) (3), large intestinal stenosis (2), elonageted sigma (2), ileostoma reversal (3), unspecified reasons (4), gall bladder carcinoma (2), small intestine carcinoma (2), chronic ileus (1), esophageal shift (1), severe motility disorder after gastrectomy (1), fistula (1), small intestine volvulus (1), small intestine perforation (1), large intestinal stoma (1) and intestinal obstruction (1). Experiments were performed in 543 mucosal/submucosal preparations 264 and 279 of which were derived from small and large intestine, respectively. All procedures were approved by the ethics committee of the Technische Universität München (1748/07 and 2595/09) with the informed patient's consent.

The preparations were from macroscopically unaffected areas as determined by the pathologists. Immediately after resection, samples were transferred to the laboratory under aseptic conditions in cold oxygenated sterile Krebs buffer. The Krebs solution contained (in mM) 117 NaCl, 4.7 KCl, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 25 $NaHCO_3$, 2.5 $CaCl_2$ and 11 glucose (all from Sigma-Aldrich, Steinheim, Germany). Transferred specimen were washed three times with ice-cold, carbogen-aerated Krebs buffer and then dissected to obtain mucosal/submucosal preparations containing the submucous plexus.

Human epithelial cell line T84

T84 cells (ECACC, Salisbury, UK) were seeded on Millipore filters (Bedford, MA, USA) with 0.45 μm pore size, and incubated at 37 °C and 95% O_2 and 5% CO_2 (Carbogen) in Dulbecco's modified Eagle medium (DMEM) / Ham's Nutrient Mixture F-12, supplemented with 10% heat-inactivated fetal calf serum, 100 IU ml-1 penicillin, 100 μg ml^{-1} streptomycin and 2.75 μg ml^{-1} amphotericin B (all from Sigma-Aldrich, Schnelldorf, Germany) to attain a monolayer. The medium was replaced daily. After 12–14 days, filters were mounted in Ussing chambers for ion transport studies. Experiments were performed on 760 T84 cell filter discs.

Ussing chamber experiments

To test the effects of STW 5 and its individual extracts on ion transport in intact human mucosal/submucosal preparations and T84 cells, we used the Ussing voltage clamp technique (Easy Mount chambers, Physiologic Instruments, San Diego, CA, USA). The tissue specimens were mounted into plexiglass Ussing chambers with exposure area of 0.5 cm^2. Mucosal and serosal sides were bathed separately in 5 ml Carbogen-bubbled Krebs solution maintained at 37 °C. The set-up allowed simultaneous measurements of up to eight mucosal/submucosal preparations dissected from one specimen. We recorded short-circuit current (I_{sc}) as a measure for the transepithelial electrogenic transport (expressed in μA/cm^2; the values were corrected for bath resistance). As previously described for the effects of STW 5 positive I_{sc} indicated a net anion current from the serosa to the lumen (Krueger et al. 2009). Tissues were electrically stimulated by silver electrodes placed on either side of the tissues and connected to a constant voltage stimulator (Grass SD-9; Astro-Med Inc., West Warwick, RI, USA). Such an electrical field stimulation (EFS) with short pulses selectively induced nerve mediated transepithelial ion fluxes. The EFS was achieved by delivering a train of pulses with supramaximal stimulus parameters: pulse amplitude, 20 V; pulse frequency 10 Hz; pulse duration 1 ms; train duration 10 s. Last but not least we measured tissue resistances.

Before starting the actual measurements, human tissues and T84 cells were allowed to equilibrate for at least 45 min or 20 min, respectively. STW 5, its individual extracts, combination of extracts and all other drugs were applied basolateraly to the serosal bathing solution except $CFTR_{inh}$-172, SITS and amiloride which were added luminally to the mucosal bathing solution.

Neither pH nor osmolality of the Krebs solution changed after addition of any of the drugs.

Drugs and herbal extracts

STW 5 (batch number E90722; Iberogast®) and its individual extracts were provided by Steigerwald Arzneimittelwerk GmbH, Darmstadt, Germany (Table 1). They belong according to the guidelines of the European Medicines Agency to "other herbal substances". The extraction processes as well as the quality controls were as previously described in detail (Kroll and Cordes 2006). Briefly, the quality of the compounds was controlled according to Good Manufacturing Practice and Good Agricultural Practice of Medicinal and Aromatic Plants (cited and outlined in Kroll and Cordes 2006). The quality of each extract is tested according to individual specification, among which is the identity of the used drug (thin layer chromatography fingerprint) and the content of marker substances (Table 1) within a defined range of ±5% measured by high performance liquid chromatography or gas-liquid chromatography.

The lyophilized extracts were dissolved in Krebs buffer and added basolaterally. We applied the individual extracts of angelica root, caraway fruit, chamomile flower, greater celandine herbs, bitter candytuft, lemon balm leaves, liquorice root, milk thistle fruit and peppermint leaves at final concentrations of 89.5, 28.4, 114.3, 62.9, 27.3, 57.9, 80.1, 14.4 and 37.2 μg/ml respectively, which corresponded to their concentrations in 512 μg/ml STW 5; this concentration evoked a reliable pro-secretory action (Krueger et al. 2009). The tenfold higher