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Effects of the herbal preparation STW 5-II on *in vitro* muscle activity in the guinea pig stomach

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Abstract

Background: STW 5 is a combination of nine medicinal herbal extracts and used to treat functional gastrointestinal disorders including functional dyspepsia. It has a region-specific effect by relaxing the proximal and contracting the distal stomach. The research combination STW 5-II (Iberogast® Advance) lacks three herbal extracts but seems clinically as effective as STW 5. However, the action of STW 5-II on gastric motility is unknown.

Methods: In vitro circular and longitudinal muscle tone and contractility were recorded from guinea pig gastric fundus and antrum with isometric force transducers.

Key Results: STW 5-II decreased concentration-dependently (64-512 µg/ml) the tone of circular and longitudinal muscle strips from the fundus. In contrast, STW 5-II increased concentration-dependently contraction amplitude in antral circular and longitudinal muscle. The effects were region-dependent but comparable in the two muscle layers. Application of 512 µg STW 5 or STW 5-II revealed comparable effects in the fundus and antrum circular and longitudinal muscle strips.

Conclusions and Interferences: STW 5-II had a region-specific effect and relaxed the proximal stomach but increased the contractility in the antrum. It was as effective as STW 5 which may explain its comparable clinical effects in treating functional dyspepsia. Impaired accommodation may be normalized through relaxation of the fundus, while the motility-promoting effects leading to an increase in antral motility may activate the gastric pump.

KEYWORDS
functional dyspepsia, gastric motility, phytotherapy, STW 5, STW 5-II

1 | INTRODUCTION

Functional dyspepsia (FD) with a global prevalence ranging between 5% and 40% is among the most widely recognized functional gastrointestinal disorders.[1,2] Besides the prevalence reported by epidemiological studies, a large proportion of patients with uninvestigated dyspepsia are eventually diagnosed as FD patients.[3] FD causes high direct and indirect health costs and thereby represents a significant financial burden on society.[4] The main symptoms of FD are postprandial fullness, early satiety, epigastric pain and burning,

nausea, vomiting, belching, heartburn, weight loss, and psychological distress related to the conditions.[1,2] Although routine diagnostic procedures do not reveal structural abnormalities, it is agreed that FD has multiple causes and several pathophysiologically relevant factors have been identified.[1,2] Among these are motility disorders such as delayed gastric emptying, impaired fundus accommodation to food, antral hypomotility, gastric hyperacidity, and psychosocial and psychosomatic factors.[1,2]

The treatment of FD is symptomatic and targets motility or secretory disorders and psychological distress and visceral hypersensitivity.[1] Among the few available options, the herbal preparation STW 5 proved to be an effective treatment by improving FD symptoms and severity scores and quality of life.[5–11]

The combination STW 5-II is a fixed combination of six hydroethanolic herbal extracts from bitter candy tuft, peppermint leaf, chamomile flower, liquorice root, caraway fruit, and melissa leaf, thereby excluding three components present in STW 5, namely extracts from angelica root, greater celandine herb, and milk thistle fruit. STW 5-II differs from STW 5 (Iberogast®) not only by the number of herbals but also by the concentration of the ingredients (Table 1). Interestingly, STW 5-II has been shown to be as effective in clinical trials as STW 5 and both were not inferior to cisapride or metoclopramide. [6,12,13] STW 5-II is introduced into the market as Iberogast® Advance in October 2020.

The mode of action of STW 5 in the stomach has been well studied in preclinical models and includes increase in antral contractility together with fundus relaxation,[14,15] and a gastro-protective action based on antisecretory, cytoprotective, and anti-inflammatory actions.[16] The effects on gastric motility remained in the presence of the neurotoxin tetrodotoxin and after functional desensitization of sensory neurons, blockade of nitric oxide pathways, or muscarinic receptors.[14] These findings strongly suggested that the effects of STW 5 on gastric motility did not require enteric nerves but were likely due to direct myogenic actions. This does not exclude a drug effect on nerves. We previously showed that the pro-secretory action of STW 5 in human intestinal mucosa/submucous plexus preparations was partly tetrodotoxin-sensitive and mainly due to activation of human submucous neurons by the angelica extract. This suggested that the neural target for STW 5 is present in submucous but not myenteric plexus.[17] STW 5 did not affect nerve-mediated responses evoked by electrical field stimulation in gastric muscle or intestinal mucosa preparations.[14,17] The STW 5 evoked relaxation in guinea pig fundus muscle was also observed in human fundus preparations.[15] Moreover, the fundus relaxation and the increased antral motility also occurred in healthy volunteers after they were given STW 5.[18] These findings emphasized the translational relevance of *in vitro* studies in guinea pig stomach.

In contrast to STW 5, the effects of STW 5-II on gastric motility are unknown. The three components lacking in STW 5-II have effects on gastric motility: Angelica root extract relaxes the fundus and contracts the antrum and thereby mimics the response to STW 5.[15] In contrast, greater celandine herb and milk thistle fruit extracts increased antral and fundus motility and may thus contribute to motility-promoting effects of STW 5 in the antrum but not on its muscle tone decreasing actions.[15] Of the remaining six extracts, liquorice root and chamomile flower mimic the effects of STW 5 on fundus and antrum.[15] We therefore hypothesize that STW 5-II, despite the lack of three components, alters gastric motility comparable to STW 5. The rationale behind this is based on the findings that the individual components of STW 5 alter antrum and fundus motility[15] and the clinical studies show similar efficacy of the two herbal preparations.[6,12]

Keypoints

- STW 5-II (Iberogast® Advance) is used to treat functional dyspepsia but its action on gastric motility is unknown.
- Therefore, we studied the effects of STW 5-II on muscle activity in isolated muscle strips from guinea pig fundus and antrum.
- STW 5-II had a region-specific effect in guinea pig stomach; it relaxed the fundus but increased the contractility in the antrum. These actions may normalize fundus accomodation as well as increase antral pump activity and may thus explain its clinical efficacy.

2 | MATERIALS AND METHODS

2.1 | Experimental procedures

This in vitro study was performed on gastric muscle preparations from 62 male guinea pigs weighing 388-588 g (Charles River Wiga, Sulzfeld, Germany). The preparation and measurements of contractility were as previously described[14] and were performed according to the German guidelines for animal protection and animal welfare and approved by the animal ethical committee of the Technical University of Munich. Briefly, after removal the stomach was opened, rinsed, and cleaned with ice cold Krebs. Muscle strips of ~1 cm^2 were cut in the longitudinal or circular direction from antrum and fundus regions. Strips were mounted in vertical organ baths (20 ml) and maintained at 37°C in carbogen-bubbled Krebs solution (in mmol/L: 117 NaCl, 4.7 KCl, 1.2 $MgCl_2$ $6H_2O$, 1.2 NaH_2PO_4, 20 $NaHCO_3$, 2.5 $CaCl_2$ $2H_2O$, and 11 glucose (all from Sigma-Aldrich, St. Louis, MO, USA). Initially, the strips were adjusted to a basal tension of 15 mN. After an equilibration period of 20 to 30 minutes, tissue viability was checked by electrical field stimulation (frequency 10 Hz, pulse duration 0.5 ms, total duration 10 s, 100 V) followed by wash out and renewal of the bathing Krebs solution. After 45-60 min, STW 5-II was added to the bathing solution at concentrations of 64, 128, 256, or 512 µg/ml, separated by wash out periods of at least 15 min. A new addition of a different concentration required that the pre-STW

TABLE 1 Composition of STW 5 and STW5-II

Medicinal Plant or Drug[a]	STW 5-II	STW 5
Caraway fruit *(Carvi fructus)*	20 ml/610 mg	10 ml/305 mg
Peppermint leaf *(Mentha piperita)*	10 ml/877 mg	5 ml/438 mg
Lemon balm leaf *(Melissae folium)*	15 ml/1050 mg	10 ml/702 mg
Chamomile flower *(Matricariae flos)*	30 ml/2020 mg	20 ml/1350 mg
Liquorice root *(Liquiritiae radix)*	10 ml/940 mg	10 ml/940 mg
Candytuft *(Iberis amara totalis)*	15 ml/265 mg	15 ml/265 mg
Angelica root *(Angelica archangelica)*	-	10 ml/1040 mg
Milk thistle fruit *(Silybum marianum L. Gaertn.)*	-	10 ml/162 mg
Greater Celandine *(Chelidonium majus)*	-	10 ml/785 mg

[a] Extraction agent for Iberis amara was 50% ethanol (V/V); for all other 30% ethanol (V/V).

5-II muscle tone was restored. Sensitization and desensitization effects were prevented by applying different concentrations in random order. As discussed before, the concentrations of STW 5-II are well below those expected in the stomach after a single therapeutic dose of 1 ml (51.3 mg/ml).[14,15,17]

STW 5-II stock solution (12.8 mg/ml) was prepared by adding Krebs solution to dry lyophilisates provided by Steigerwald Arzneimittel GmbH, Darmstadt, Germany, and stored at +4°C in a desiccator. The stock solution was renewed every three days. The therapeutic dose of STW 5-II is 1 ml (20 drops) 3 times daily.

The experiments to compare the strength of the effects of STW 5-II and STW 5 were performed on muscle strips taken from immediately adjacent regions from the same stomach, on the same day, under the same conditions (see Table 1 for composition of STW5 and STW 5-II).

2.2 | Data and statistical analysis

All values are presented as medians and their 25% and 75% quartiles (given in brackets in the text). The statistical tests were carried out with Sigmastat 3.10 (Systat Software Inc, Erkrath, Germany). Results were considered significant for p-values <0.05. The motility traces were recorded and analyzed with the Chart 4.2 software (ADInstruments, Spechbach, Germany). For the analysis of differences in fundus muscle tone, the average mN values during 2 min periods just before adding STW 5-II or STW 5 and during the maximal response to either of the drugs were calculated as in our previous study.[14] Antrum motility was analyzed by calculating the average contraction amplitude during 2 min periods just before adding STW 5-II or STW 5 and during the maximal response to either drug as in our previous study.[14] The experimental protocol was as such that N numbers of tissues always reflected number of animals.

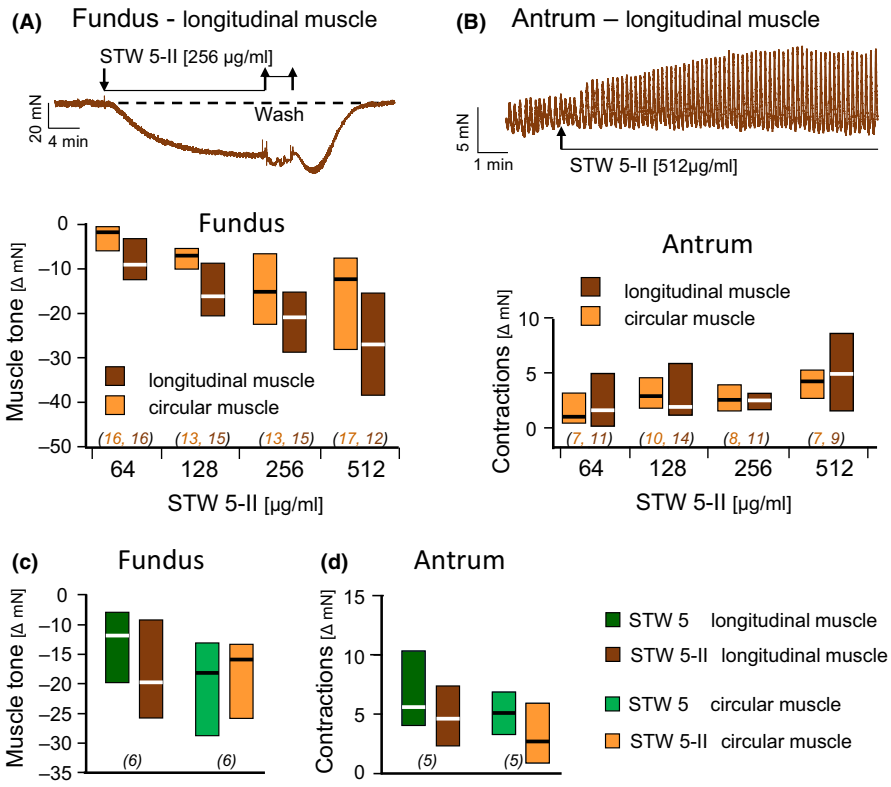

FIGURE 1 STW 5-II concentration-dependently decreased muscle tone in the proximal but increase motility in the distal stomach. (A) Upper panel is a representative trace showing the decrease in fundus muscle tone after application of STW 5-II (downward arrow). The muscle tone fully recovered after wash out (upward arrows). Below is the summary graph showing the concentration-dependent relaxation in longitudinal and circular muscle strips from fundus. (B) Upper panel is a representative trace showing the increased contractile amplitude in antral muscle strips after application of STW 5-II. STW 5 and STW 5-II, both at 512 µg/ml, evoked comparable relaxation of muscle strips in the fundus (C) and similar motility-promoting actions in the antrum (D). *N* numbers are given in parenthesis

2.3 | Animal welfare statement

All animal work was conducted according to the German guidelines for animal care and welfare (Deutsches Tierschutzgesetz) and approved by the Bavarian state ethics committee (Regierung Oberbayern, which serves as the Institutional Care and Use Committee for the Technische Universität München) according to §4 and §11 Deutsches Tierschutzgesetz under reference number 32-568-2.

3 | RESULTS

3.1 | Effects of STW 5-II on gastric motility

STW 5-II relaxed both the circular and longitudinal muscle preparations of the fundus (Figure 1A). The relaxation was sustained till wash out of STW 5-II (Figure 1A). The decrease in muscle tone was concentration dependent and significant at all concentrations (Figure 1A, p = 0.0001-0.01 for circular and p = 0.0002-0.006 for longitudinal muscle). There was no difference in the degree of relaxation between circular as well as longitudinal muscle (Figure 1A, p = 0.08-0.24).

In the antrum, STW 5-II increased contractile amplitude (Figure 1B). These motility-promoting effects were concentration dependent and significant at all concentrations (Figure 1B, p = 0.003-0.03 for circular and p = 0.001-0.02 for longitudinal muscle). The enhancement of the contraction amplitude was comparable between the two muscle layers (Figure 1B, p = 0.5-1).

3.2 | Comparison between effects of STW 5-II and STW 5 on the gastric motility

Comparing results from our previous study[14] with the present one suggested that the effects of STW 5 and STW 5-II on muscle tone in the proximal and contractile activity in the distal stomach were comparable. We wanted to know whether this impression was true and performed paired experiments with a concentration of 512 µg/ml for both drugs. We used preparations from the same guinea pig and applied either STW 5 or STW 5-II. In muscle strips from the fundus, both drugs evoked almost identical relaxations with no differences in the amplitude of the response between circular and longitudinal muscle (Figure 1C). Likewise, both drugs enhanced antral contraction amplitude almost to the same extent; the circular and longitudinal muscle layers responded equally (Figure 1D).

The basal contraction frequency was 4.6 ± 0.3 per min and 4.8 ± 0.2 per min in the circular and longitudinal muscle, respectively, and remained more or less unchanged in STW 5 or STW 5-II. The difference in the frequency was 0.01 Hz or 0.0 Hz for STW 5 and STW 5-II (for both n = 5) in the circular muscle and 0.02 Hz for both STW 5 and STW 5-II (for both n = 5) in the longitudinal muscle.

Both the relaxation in the fundus and the increase in contraction amplitude in the antrum started within 1-2 min after application of STW 5 or STW 5-II. The maximal decrease in muscle tone was reached for circular 32 min [10/96] or 32 min [11/93] after STW 5 or STW 5-II (n = 5; p = 0.2), respectively, and for longitudinal muscle 15 min [9/49] or 25 min [12/44] after STW 5 or STW 5-II (n = 5, p = 0.3), respectively. The maximal increase in contraction amplitude in the antrum was observed for circular muscle 12 min [4/13] or 7 min [1/36] after STW 5 or STW 5-II (n = 6, p = 0.6), respectively, and for longitudinal muscle 5 min [2/20] or 3 min [2/7] after STW 5 or STW 5-II (n = 6, p = 0.2), respectively.

4 | DISCUSSION

The results of our study suggested that STW 5-II relaxed smooth muscle in the fundus but enhanced contraction amplitude in the antrum. These region-specific but layer-independent effects were identical to those described for STW 5.[14] This was confirmed by dedicated experiments in the present study comparing the effects of STW 5 and STW 5-II. Thus, the muscle-relaxing effects in the proximal stomach and the contraction-enhancing effects in the antrum were similar for the two drugs. We believe that both drugs act on circular and longitudinal muscle but cannot rule out that contraction of the longitudinal muscle could cause passive interactions with the circular muscle and vice versa.[19]

Our findings provide a mechanistic rational for the efficacy of STW 5-II in the treatment of FD patients.[6,12,13] The fundus region serves to store the food and adapts its volume to the meal size. The muscle-relaxing effects of STW 5-II in the fundus should improve this accommodation reflex which was shown to be impaired in FD patients.[20] Functional dyspepsia is also associated with antral hypomotility.[21] The stimulating effect of STW 5-II in the antrum may improve the impaired antral motility.

It is noteworthy that STW 5-II inhibitory effects on muscle tone in the fundus were as strong as those of STW 5 although STW 5-II lacked angelica, one component that evoked a strong relaxation of the fundus.[15] An explanation for the comparable inhibitory effects on the proximal stomach motility may be the lack of the contractile action of greater celandine (not present in STW 5-II) together with a higher concentration of chamomile flower in STW 5-II, which had a potent muscle-relaxing effect.[15] The higher concentration of lemon balm and caraway may explain the still strong increase in antral motility by STW 5-II. Both extracts increased contractile amplitude in the antrum and may compensate for the lack of angelica, greater celandine, and milk thistle extracts which all increased antral motility.[15]

In summary, our study revealed region-specific effects of STW 5-II in guinea pig stomach *in vitro*, a concentration-dependent relaxation of the fundus but increased antrum contractility. This action of STW 5-II resembles that of STW 5 and may explain the beneficial effect of STW 5-II in FD patients. Impaired accommodation may be normalized through relaxation of the fundus, while the motility-promoting effects leading to an increase in antral motility may activate the gastric pump.

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CONFLICT OF INTEREST

Ramy M. Ammar and Olaf Kelber are employees of Bayer Consumer Health, Steigerwald Arzneimittelwerk GmbH, and Darmstadt. All other authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

ML, DK, and OK performed experiments and data analysis, KM performed statistical analysis, MS designed and finalized manuscript, RA drafted manuscript, and all authors accepted final manuscript.

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