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Binding of STW 5 (Iberogast®) and its components to intestinal 5-HT, muscarinic M_3, and opioid receptors

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Abstract

Clinical studies with the fixed herbal combination product STW 5 (Iberogast®) have indicated an efficacy comparable to metoclopramide (5-HT_3 antagonist) and cisapride (5-HT_4 agonist) in functional gastro-intestinal diseases like functional dyspepsia (FD) and irritable bowel syndrome (IBS). Since serotonin (5-HT_3 and 5-HT_4) and muscarinic M_3 receptors are known to play a central role in the etiology of FD and IBS, the extracts contained in STW 5 and several of their phytochemical components were studied in vitro for binding affinities to these receptors of the intestine. STW 5 inhibited the binding of ^{3}H-GR113808 and ^{3}H-4-DAMP to 5-HT_4 and M_3 receptors, respectively, about 10 times more potently than the binding of ^{3}H-GR65630 to 5-HT_3 receptors. IC_{50} values for STW 5 did correspond to extract dilutions of 1:1000 (M_3 binding) and 1:2000 (5-HT_4 binding). In addition, STW 5 also potently inhibited the binding to opioid receptors with an IC_{50} value of 1:2000. Of the nine herbal extracts contained in STW 5, the fresh plant extract of bitter candy tuft (*Iberis amara*) selectively inhibited binding to M_3 receptors, while ethanolic extracts of celandine herb and chamomile flower were selective to 5-HT_4, and liquorice root to 5-HT_3 receptors. Binding affinities to human recombinant 5-HT_3, 5-HT_4 and M_3 receptors were qualitatively similar to those of the corresponding intestinal receptors. The benzylisoquinoline alkaloid berberine had significant inhibitory action on 5-HT_4 and M_3 binding, showing IC_{50} values of 40 ng/ml (100 nM) and 200 ng/ml (500 nM), respectively, but is present in the extract of celandine herb only in traces, so that also for the celandine extract a cooperative effect of several phytochemical constituents can be assumed. These in vitro data indicate that 5-HT_4 (to a lesser degree 5-HT_3), muscarinic M_3, and opioid receptors represent target sites for the treatment of FD and IBS with STW 5 (Iberogast®).

Keywords: Receptor binding; Serotonin 5-HT_3; 5-HT_4; Muscarinic M_3; Opioid receptors; Functional dyspepsia (FD); Irritable bowel syndrome (IBS); STW 5; Iberogast; *Iberis amara*; *Chamomila recutita*; *Mentha piperita*; *Carum carvi*; *Chelidonium majus*; *Silybum marianum*; *Angelica archangelica*; *Glycyrrhiza glabra*; *Melissa officinalis*

Introduction

The fixed herbal product STW 5 (Iberogast®) is composed of 15 ml of a fresh plant extract (1:2, 50% v/v ethanol) of bitter candy tuft and extracts (1:3, 30% v/v ethanol) of angelica root (10 ml), milk thistle fruit

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(10 ml), caraway fruit (10 ml), celandine herb (10 ml), liquorice root (10 ml), chamomile flower (20 ml), lemon balm leaf (10 ml) and peppermint leaf (5 ml) per 100 ml. It has been clinically proven to be effective against non-ulcer dyspepsia and irritable bowel syndrome (Saller et al., 2002; Rösch et al. 2006) with similar efficacy but less side effects as cisapride (Roesch et al., 2002) and metoclopramide (Nikolay, 1984).

In pharmacological studies with the isolated guinea pig ileum, stimulated with acetylcholine or histamine, STW 5 has been shown to exhibit spasmolytic activities as well as the extracts of chamomile flowers, liquorice root, and peppermint leaves (Ammon et al., 2006). In the non-stimulated ileum preparation STW 5 and its component *Iberis amara* raised the basal tone of the smooth muscles (Okpanyi et al., 1993; Ammon et al., 2006; Michael et al., 2006). In the stomach, a region specific effect was described in vitro (Schemann et al., 2006), which has been confirmed in a clinical pharmacological study, too (Pilichiewicz et al., 2006). Besides these motility-related effects an effect on gastro-intestinal sensitivity (Müller et al., 2006) and a dose dependent anti-ulcerogenic activity as well as cytoprotective effects were shown in in vivo studies (Khayyal et al., 2006). The uptake of characteristic constituents of the extracts by the small intestine has been proven in an ex vivo model (Kelber et al., 2006).

Functional gastro-intestinal diseases like functional dyspepsia (FD) and irritable bowel syndrome (IBS) are some of the most common ailments for medical treatment in industrialized countries (Allescher, 2006). Synthetic drugs like alosetron (5-HT_3 antagonist), cisapride and tegaserod (both 5-HT_4 agonists) have recently been developed. But despite promising pharmacological evidence, their clinical use remains restricted: Alosetron and cisapride have been removed from the market, because of severe adverse effects like ischemic colitis and cardiac arrhythmias (Callahan, 2002; Kamm, 2002). However, cisapride has recently been reapproved under restrictions in certain countries. Whether the partial agonist tegaserod can prevail as clinical remedy, remains to be seen. Nevertheless, there is significant pharmacological evidence that 5-HT_3 and 5-HT_4 receptors of the submucosal sensory neurons as well as the muscarinic M_3 receptor of smooth muscle cells represent promising targets for the treatment of functional gastro-intestinal diseases (Houghton et al., 1997; Prins et al., 1999; Berman et al., 2002). Moreover, opioid receptor agonists have also been discussed as new drugs in the treatment of FD (Stanghellini et al., 2003; Camilleri 2004). Since the effect of STW 5 on these intestinal receptors had not been studied yet, competitive binding experiments with STW 5 components and its characteristic constituents were performed.

Materials and methods

Ethanolic extracts (30% v/v ethanol) of STW 5 were diluted with H_2O to prepare the following extract dilutions studied in the binding experiments: 1:200, 1:500, 1:1000, and 1:10,000. Cisapride was purchased from RDI (Flanders, NJ, USA). Intestine of the rat (Wistar, RCC Ltd., Switzerland) was homogenized in 50 mM Tris/HCl pH 7.4 on ice using a Polytron homogenizer. The homogenate was centrifuged at 38,000*g* for 20 min and washed five times. The preparation was stored as a pellet at −80 °C and was homogenized each time when used for binding studies. The protein content of the intestine for each binding assay was about 20 μg/ml as determined by the BCA protein assay.

Binding assays to 5-HT_3, 5-HT_4, muscarinic M_3, 5-HT_{1A}, and opioid receptors of the intestine were performed with ^{3}H-GR65630 (5-HT_3 antagonist, 0.2 nM), ^{3}H-GR113808 (5-HT_4 antagonist, 0.2 nM), (N-methyl-^{3}H) 4-DAMP (M_3 antagonist, 0.2 nM), ^{3}H-8-OH-DPAT (5-HT_{1A} agonist, 0.5 nM), and ^{3}H-naloxone (non-selective antagonist, 3.6 nM), while non-specific binding was determined in the presence of metoclopramide, 5-HT, atropine, 8-OH-DPAT (each 10^{-4} M), and naloxone (2×10^{-5}), respectively. Binding equilibration was terminated by filtration (Whatman GF/C filter) and radioactivity was quantified by liquid scintillation counting. IC_{50} values expressed as extract dilution were graphically determined according to concentration response curves of the specific binding (difference between specific and non-specific binding in the presence of given extract concentrations).

Results and discussion

STW 5 and its nine herbal component extracts affected the binding to 5-HT_3, 5-HT_4 and muscarinic M_3 with different potencies as summarized in Table 1. STW 5 showed a >10 fold higher affinity to both 5-HT_4 and M_3 receptors than to 5-HT_3 receptors. IC_{50} values for the fixed combination STW 5 did correspond to dilutions of 1:2000 (5-HT_4 binding) and 1:1000 (M_3 binding). Whether these pharmacological actions on 5-HT_4 and M_3 receptors account for the observed effects in vivo, cannot directly be concluded. Considering a dilution factor of approximately 1:1000 for STW 5 extracts entering the stomach (1 ml drug in 1 l gastro-intestinal volume), in vivo effects seem to be plausible for seven of the nine component extracts that showed IC_{50} values $<$1:1000. Among the nine herbal extracts, the fresh plant extract of *bitter candy tuft* selectively inhibited binding to M_3 receptors, while ethanolic extracts of celandine herb and chamomile flowers were

Table 1. Binding affinities of STW 5 and its components to rat intestinal 5-HT_3, 5-HT_4 and muscarinic M_3 receptors expressed as IC_{50} values

	IC_{50} (extract dilution)		
	5-HT_3	5-HT_4	M_3
Iberogast® (STW 5)	<1:100	1:2000	1:1000
Peppermint leaf (STW 5-KII)	—[a]	1:2000	—[b]
Chamomile flower (STW 5-KIII)	—[a]	1:10,000	—[b]
Liquorice root (STW 5-KIV)	1:2000	1:500	—[b]
Angelica root (STW 5-KV)	1:2000	1:2000	1:1250
Caraway fruit (STW 5-KVI)	—[a]	—[a]	—[b]
Milk thistle fruit (STW 5-KVII)	—[a]	—[a]	—[b]
Melissa leaf (STW 5-KVIII)	<1:100	1:1000	—[b]
Celandine herb (STW 5-KIX)	1:1000	>1:10,000	1:3500
Bitter candy tuft (STW 6)	<1:100	1:500	1:1500

[a]High non-specific binding in the presence of extract unables determination of IC_{50} value.

[b]Specific binding was inhibited <20% in the presence of extract dilutions of 1:2000.

Table 2. Binding affinities of STW 5 and components to human recombinant 5-HT_3, 5-HT_4 and muscarinic M_3 receptors expressed as IC_{50} values

	IC_{50} (extract dilution)		
	5-HT_3	5-HT_4	M_3
Iberogast® (STW 5)	1:50	1:160	1:125
Liquorice root (STW 5-KIV)	1:10	n.d.	n.d.
Celandine herb (STW 5-KIX)	n.d.	1:330	n.d.
Bitter candy tuft (STW 6)	n.d.	n.d.	<1:100

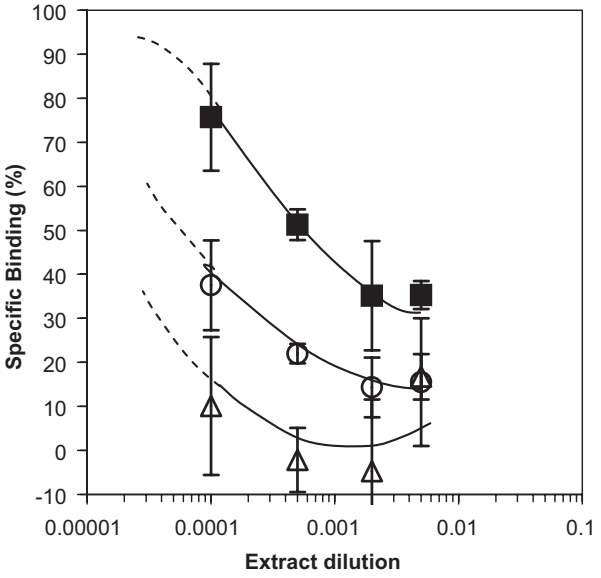

Fig. 1. Competition for ^{3}H-GR113808 binding to intestinal 5-HT_4 receptors in the presence of STW 5 (■), chamomile flower (○) and celandine herb (△). Data are given as mean values ± S.D. of three replicates.

Table 3. Binding affinities of STW 5 to rat intestinal 5-HT_{1A} and opioid receptors expressed as IC_{50} values

	IC_{50} (extract dilution)	
	5-HT_{1A}	Opioid (non-selective)
STW 5 (Iberogast®)	—[a]	1:2000

[a]No inhibition at extract dilutions between 1:2000 and 1:500.

selective to 5-HT_4, and liquorice roots to 5-HT_3 receptors. For caraway and milk thistle fruit extracts, no receptor affinity could be detected. These two extracts exhibited high non-specific binding making an IC_{50} determination impossible. Such interfering effects can usually be prevented by elimination of the corresponding compounds via extract fractionation. It is therefore possible that parts of these extracts lacking these interfering constituents may also show binding affinity to the receptors studied.

STW 5 and selected component extracts showed qualitatively similar binding affinities to human recombinant 5-HT_3, 5-HT_4 and muscarinic M_3 receptors as to the corresponding intestinal receptors (Table 2). The binding affinities of STW 5, extracts of liquorice roots, celandine herb and *Iberis amara* to the recombinant receptors were, however, approximately 10 times lower than to the intestinal receptors, probably due to a lower specific binding of the tritiated ligands.

Specific binding of STW 5 and its component extracts chamomile flowers and celandine herb to 5-HT_4 receptors is shown in Fig. 1. Four extract dilutions (1:200, 1:500, 1:2000, and 1:10,000) were tested at each case to graphically determine IC_{50} values. Both extracts evidently inhibited the binding of ^{3}H-GR113808 more potently than the other components of STW 5 indicating their relevance in the fixed herbal combination.

STW 5 also potently inhibited the non-selective binding of ^{3}H-naloxone to intestinal opioid receptors with an IC_{50} value of 1: 2000 (Table 3). No binding inhibition up to a dilution of 1:500 was observed to the intestinal 5-HT_{1A} receptor.

Binding affinities of characteristic constituents of STW 5 components to 5-HT_4 and M_3 receptors are summarized in Table 4. Beside berberine chloride, none of the constituents tested significantly inhibited ^{3}H-GR113808 and (*N*-methyl-^{3}H) 4-DAMP binding. Berberine chloride inhibited with similar potency as the reference 5-HT_4 agonist cisapride. Whether berberine also exhibits an action in vivo is doubtful, since the benzylisoquinoline alkaloid is present in extracts of celandine herb (STW 5-K IX) only in traces (total

Table 4. Binding affinities of STW 5 constituents to rat intestinal 5-HT_4 and M_3 receptors expressed as IC_{50} values

	IC_{50} (μg/ml)	
	5-KHT_4	M_3
Cucurbitacin E[1]	>20	>10
Cucurbitacin I[1]	>20	10
Glucoiberin[1]	>20	>10
Kaempferol-3,4-β-diglucopyranoside-7-O-rhamnopyranoside[1]	>20	>10
α-Bisabolol[2]	>20	n.d.
Matricine[2]	>20	n.d.
Chamazulene[2]	>20	n.d.
N-malonyltryptophan[3]	>20	>10
Chelidonic acid[4]	>20	>10
Chelidonine[4]	>20	10
Berberine chloride[4]	0.04	0.2
Sparteine[4]	n.d.	10
Osthole[5]	n.d.	>10
Angelicine[5]	n.d.	>10
Bergaptene[5]	n.d.	10
Psoralene[5]	n.d.	10
Chlorogenic acid[5]	n.d.	10
Umbelliferone[5]	n.d.	10
Angelicalactone[5]	n.d.	10
Cisapride	0.025	
Atropine	0.07	

Compound contained in extracts of [1]Bitter candy fruit, [2]Camomile flower, [3]Milk thistle fruit, [4]Celandine herb, [5]Angelica root; n.d. not determined.

alkaloid content <0.8 mg/g). Since none of the remaining characteristic constituents of celandine herb (chelidonine and chelidonic acid) and chamomile flowers (α-bisabolol, matricine and its degradation product chamazulene) inhibited the binding on 5-HT_4 and M_3 receptors, other constituents seem to account for the observed effects of the extracts. The phenomenon that single compounds fail to explain the effect of complex herbal mixtures seems to be a common case in such in vitro test systems. An explanation for this phenomenon may be that weak effects of various constituents interact in an unknown manner (e.g. additive, competitive, synergistic, etc.), which has to be looked further into in future studies.

Conclusions

Iberogast® (STW 5) has been shown to exhibit binding affinity to intestinal 5-HT_3, 5-HT_4, muscarinic M_3, and opioid receptors with a >10 fold preference for the three latter receptor types. The comparison of the nine herbal component extracts present in STW 5 with regard to binding affinity to these receptors revealed a different pattern of selectivity. Whereas a fresh plant extract of *Iberis amara* selectively inhibited binding to M_3 receptors, ethanolic extracts of celandine herb and chamomile flowers were selective to 5-HT_4, and liquorice roots to 5-HT_3 receptors. These findings indicate the involvement into receptor binding of seven of the nine components of STW 5 used for functional gastro-intestinal diseases like non-ulcer dyspepsia and irritable bowel syndrome. In conclusion, our in vitro data confirm the relevance of the components of STW 5 for its efficacy in the treatment of functional gastro-intestinal diseases acting via crucial target sites including 5-HT_3, 5-HT_4, muscarinic M_3, and opioid receptors.

References

Allescher, H., 2006. Functional dyspepsia – a multi-causal disease and its therapy. Phytomedicine, this issue.

Ammon, H.P.T., Kelber, O., Okpanyi, S.N., 2006. Spasmolytic and tonic effect of Iberogasts (STW 5) in intestinal smooth muscle. Phytomedicine, this issue.

Berman, S.M., Chang, L., Suyenobu, B., Derbyshire, S.W., Stains, J., Fitzgerald, L., Mandelkern, M., Hamm, L., Vogt, B., Naliboff, B.D., Mayer, E.A., 2002. Condition-specific deactivation of brain regions by 5-HT3 receptor antagonist Alosetron. Gastroenterology 123, 969–977.

Callahan, M.J., 2002. Irritable bowel syndrome neuropharmacology. A review of approved and investigational compounds. J. Clin. Gastroenterol. 35 (Suppl.), 58–67.

Chamilleri, M., 2004. Treating irritable bowel syndrome: overview, perspective and future therapies. Br. J. Pharmacol. 141, 1237–1248.

Houghton, L.A., Rogers, J., Whorwell, P.J., Campbell, F.C., Williams, N.S., Goka, J., 1997. Zamifenacin (UK-76, 654) a potent gut M3 selective muscarinic antagonist, reduces colonic motor activity in patients with irritable bowel syndrome. Aliment Pharmacol. Ther. 11, 561–568.

Kamm, M.A., 2002. The complexity of drug development for irritable bowel syndrome. Aliment Pharmacol. Ther. 16, 343–351.

Kelber, O., Wittwer, A., Lapke, C., Weiser, D, Okpanyi, S.N., Heilmann, J., 2006. Absorption of STW 5 (Iberogast) and its components ex vivo/in vitro. Phytomedicine, this issue.

Khayyal, M.T., Seif-El-Nasr, M., El-Ghazaly, M.A., Okpanyi, S.N., Kelber, O., Weiser, D., 2006. Mechanisms involved in the gastro-protective effect of STW 5 (Iberogast®) and its components against ulcers and rebound acidity. Phytomedicine, this issue.

Michael, S., Kelber, O., Vinson, B., Nieber, K., 2006. Herbal preparations STW 5 and STW 6 inhibit inflammation-mediated motility disorders in the ileum. Gut, in press.

Müller, M.H., Liu, C.-Y., Glatzle, J., Weiser, D., Kelber, O., Enck, P., Grundy, D., Kreis, M.E., 2006. STW 5 (Iberogast) reduces afferent sensitivity in the rat small intestine. Phytomedicine, this issue.

Nikolay, K., 1984. Funktionelle Gastroenteropathien im therapeutischen Blindvergleich von Metoclopramid mit dem Phytopharmakon Iberogast. Gastro-Entero-Hepatologie 2, 24–28.

Okpanyi, S.N., Mark, Wahl, M.A., 1993. Gastrointestinal motility modulation with Iberogast. Acta Hortic. 332, 227–235.

Prins, N.H., Van Haselen, J.F., Lefebvre, R.A., Briejer, M.R., Akkermans, L.M., Schuurkes, J.A., 1999. Pharmacological characterization of 5-HT4 receptors mediating relaxation of canine isolated rectum circular smooth muscle. Br. J. Pharmacol. 127, 1431–1437.

Pilichiewicz, A.N., Horowitz, M., Russo, A., Maddox, A.F., Jones, K.L., Schemann, M., Holtmann, G., Feinle-Bisset, C., 2006. Effects of Iberogast® on proximal gastric volume, antropyloroduodenal (APD) motility and gastric emptying in healthy men. Neurogastroenterol. Motility, in press.

Roesch, W., Vinson, B., Sassin, I., 2002. A randomised clinical trial comparing the efficacy of a herbal preparation STW 5 with the prokinetic drug cisapride in patients with dysmotility type of functional dyspepsia. Z. Gastroenterol. 40, 401–408.

Rösch, W., Vinson, B., Gundermann, K.H., Holtman, G., 2006. Clinical efficacy and safety of STW 5 – a review. Phytomedicine, this issue.

Saller, R., Pfister-Hotz, G., Iten, F., Melzer, J., Reichling, J., 2002. Iberogast: Eine moderne phytotherapeutische Arzneimittelkombination zur behandlung funktioneller Erkrankungen des Magen-Darm-Trakts (Dyspepsie, Colon irritabile) – von der Pflanzenheilkunde zur ''Evidence Based Phytotherapy''. Eine systematische Übersicht. Forsch Komlementärmed Klass Naturheilk 9 (Suppl. 1), 1–20.

Schemann, M., Hohenester, B., Rühl, A., 2006. Region-specific effects of STW 5 and its components in gastric fundus/corpus and antrum. Phytomedicine, this issue.

Stanghellini, V., De Ponti, F., De Giorgio, R., Barbara, G., Tosetti, C., Corinaldesi, R., 2003. New developments in the treatment of functional dyspepsia. Drugs 63, 869–892.