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COMMENTARY

Can small non-peptide motilin agonists force a breakthrough as gastroprokinetic drugs?

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GSK962040 is a small selective motilin receptor agonist currently under investigation in clinical trials for the treatment of conditions associated with delayed gastric emptying. As reported in this issue of the *British Journal of Pharmacology*, Broad *et al.*, studied for the first time the region-dependent contractile effects of motilin and GSK962040 in human smooth muscle strips. Both compounds facilitated cholinergically mediated contractions of human gastric antral muscle strips at low concentrations and induced smooth muscle contractions at high concentrations. The effect was less pronounced in the fundus and almost absent in the colon. The long-lasting facilitation of cholinergic responses in the antrum by GSK962040 compared with the fading responses to motilin may be of importance from a clinical point of view. The approach used by Broad *et al.* with human tissue is a validated model to identify motilin receptor agonists with therapeutic value.

LINKED ARTICLE
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Abbreviations
EGFP, enhanced green fluorescent protein; MMC, migrating motor complex; MTLR, motilin receptor

The finding that the macrolide antibiotic erythromycin A is a motilin agonist that stimulates gastric emptying in patients with diabetic gastroparesis has stimulated the development of motilin agonists with the aim of generating a new class of gastroprokinetic agents (Peeters *et al.*, 1989; Janssens *et al.*, 1990). The first motilides, macrolide compounds without antibacterial activity but with motilin agonist activity, were developed in the early 1990s. Several large clinical trials with one of the lead compounds, ABT-229, were conducted, but the further development of the compound was stopped because ABT-229 failed to relieve symptoms in patients with functional dyspepsia (Talley *et al.*, 2000) and diabetic gastroparesis (Talley *et al.*, 2001).

After these negative trials, the development of motilin agonists with the complex macrolide structure was abandoned. Recently, a second generation of synthetic small non-peptide motilin agonists has been developed first by Bristol-Myers Squibb, for example, BMS-591348 (Li *et al.*, 2004) and later on by GlaxoSmithKline, for example, GSK962040 (Westaway *et al.*, 2009) and RaQualia Pharma, for example, RQ-00201894 (Takahashi *et al.*, 2010).

In general, studies on the mechanism of action of motilin have been hampered by the absence of a functional motilin receptor in the gastrointestinal tract of rodents. Nevertheless, many studies have been performed with motilin in other species. Initial studies were mainly focused on the smooth muscle effects of motilin in muscle strips from human, rabbit, cat and chicken (see De Smet *et al.*, 2009). Neural responses to motilin were demonstrated, for the first time, with electrical field stimulation of strips of the chicken proventriculus (Kitazawa *et al.*, 1995), and later on with strips from the rabbit antrum (Van Assche *et al.*, 1997; Dass *et al.*, 2003). The existence of motilin receptors located on nerves or smooth muscle-enriched preparations from human or rabbit tissue was further confirmed by receptor-binding studies (Van Assche *et al.*, 1998; Miller *et al.*, 2000).

The region-dependent contractile efficacy of motilin and the motilin agonist, GSK962040, was for the first time studied

in detail *in vitro* in human intestinal tissue by Broad *et al*. (2012) and is reported in the current issue of *BJP*. The authors confirmed previous findings by Van Assche *et al*. (1997) in the rabbit antrum that motilin enhanced cholinergic neurotransmission at low doses and interacted directly with antral smooth muscle receptors at high doses. Similarly, in healthy volunteers, a low dose of erythromycin A was shown to induce a premature antral activity front via activation of a cholinergic pathway and an atropine-resistant, non-propagating, contraction of prolonged activity, probably involving a muscular contraction at higher concentrations (Coulie *et al*., 1998).

Thielemans *et al*. (2002) showed that the potency of peptidyl and non-peptidyl motilin agonists to induce Ca^{2+} responses in CHO cells expressing the cloned human motilin receptor (CHO-MTLR cells) correlated with the potency to induce smooth muscle responses in the rabbit intestine, indicating that the motilin pharmacophore is well conserved among species. Dass *et al*. (2003) confirmed that the rabbit motilin receptor is a close orthologue of the human receptor in terms of sequence identity. All these studies suggest that the rabbit isolated stomach preparation is the best animal model to screen for motilin agonists with prokinetic potential in humans.

Dyspeptic symptoms have a heterogeneous origin and besides delayed gastric emptying and hypersensitivity to gastric distension, impaired accommodation to a meal seems to be an important pathophysiological mechanism.

Fundic contractions may impair accommodation and may have contributed to the worsening of symptoms observed in the clinical trial with ABT-229. Indeed, Cuomo *et al*. (2006) showed that infusion of motilin increased fasting fundic tone, reduced gastric accommodation and increased meal-induced satiation. The effect was not blocked by atropine, suggesting that motilin induces proximal stomach contraction via a receptor primarily located on smooth muscle. The study of Broad *et al*. (2012) investigated for the first time the effect of motilin on fundic contractions *in vitro* and found that, at low concentrations, motilin and GSK962040 facilitated the contractions elicited by electrical field stimulation. Interestingly, the smooth muscle response to GSK962040 was only half of the response to motilin in the fundus. This suggests that GSK962040 is less likely than motilin to affect fundic tone *in vivo* and to impair accommodation. In the antrum, the GSK compound was even without effect on the smooth muscle.

Previous studies investigating the effect of motilin or erythromycin A on the contractility of human colonic smooth muscle strips and on Ca^{2+} signalling in colonic smooth muscle cells in culture showed that motilin exerted weak direct excitatory smooth muscle effects (Van Assche *et al*., 2001). These observations were confirmed by Broad *et al*., but in addition the authors showed that motilin did not facilitate neural responses in the colon and that GSK962040 was without effect in the colon. It is therefore unlikely that the GSK compound can be used for the treatment of constipation. However, *in vivo* studies are needed to confirm this.

An important finding of the current study by Broad *et al*. (2012) was that cholinergic facilitation by motilin in the antrum faded, whereas facilitation by GSK962040 was long lasting. Similar observations were made with motilin in the rabbit antrum (Dass *et al*., 2003). Fading of the response to motilin may be consistent with its physiological role in the induction of phase 3 activity of the migrating motor complex (MMC). It has been shown that during phase 1 of the MMC, the human and canine small intestine is refractory to the action of motilin. Such a phenomenon could be explained by a down-regulation of motilin receptors caused by the rise in plasma motilin levels which accompanies phase 3.

The motilin receptor follows the desensitization paradigm operative for most GPCR: phosphorylation of the receptor, recruitment of β-arrestin-2 which targets the receptor to clathrin-coated pits, internalization, dephosphorylation and ligand dissociation, sorting to the recycling endosomes and trafficking of the receptor back to the plasma membrane (Lamian *et al*., 2006; Mitselos *et al*., 2008).

The desensitization of the motilin receptor, together with the wrong dosage selection, has been considered as an important reason for the clinical failure of ABT-229. In CHO-MTLR cells with enhanced green fluorescent protein (EGFP), ABT-229 was a much more potent inducer of desensitization than motilin, EM-A and other motilides, because the compound induced a higher degree of internalization and delayed the recycling of the internalized receptors to the plasma membrane (Thielemans *et al*., 2005; Mitselos *et al*., 2008). An important feature of the GSK compound studied by Broad *et al*. (2012) was its long-lasting neural activity compared with motilin. It was suggested by the authors that GSK962040 may bind to a different site on the receptor from that used by motilin and, in this way, influence other downstream mechanisms. However, it is much more likely that GSK962040 may be less prone to induce desensitization of the motilin receptor. This is in line with previous observations showing that not all motilin agonists have the same desensitizing properties and that some compounds, for example, ABT-229, have a stronger desensitizing potency than would be expected on the basis of their activity (Thielemans *et al*., 2005). It would be interesting to compare the desensitizing but also the resensitizing properties of GSK962040 with those of motilin and ABT-229 in the *in vitro* CHO-MTLR-EGFP trafficking model of Mitselos *et al*. (2008). This would definitively validate the usefulness of the current *in vitro* model used by Broad *et al*. (2012) to screen for motilin agonists with clinical benefit in which the facilitating effect of the motilin agonist on gastric neuronal activity represents a model to test the activity of the compound, while the fading of the response would represent a measure for the desensitizing properties of the agonist.

In summary, GSK962040 seems to meet the criteria of an ideal motilin agonist. At low doses, the compound maximally activates gastric neuronal motilin receptors with no smooth muscle effects in the fundus which may impair accommodation. In addition, the long-lasting facilitation of the neural response by GSK962040 suggests that the compound does not induce receptor desensitization. Oral administration of GSK962040 as single or repeated doses accelerated gastric emptying in a maintained manner in healthy volunteers (Dukes *et al*., 2009; 2010). Preliminary results from phase 2 studies investigating the effect of single oral doses of GSK962040 for the treatment of gastroparesis in type 1 diabetes indicate that the compound is well tolerated and increases gastric emptying (Hellstrom *et al*., 2011). A similar study is going on in critically ill patients with enteral feed

intolerance. The safety, efficacy and dose response of 28 days of once-daily oral dosing of GSK962040 will soon be tested in (type 1 or type 2) diabetic patients with gastroparesis.

These studies with GSK962040 could force a breakthrough for the further development of motilin receptor agonists with gastroprokinetic potential. In the future, the most important competitor of GSK962040 may be the oral ghrelin agonist, TZP-102, which has been tested in phase 2 studies in patients with diabetic gastroparesis with positive effects on both symptoms and gastric emptying rate (McCallum *et al.*, 2011). After many years of pioneering work, the future will show which one of the two cognate peptides will receive the label of a new gastroprokinetic drug.

Conflict of interest

None.

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