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MOTILIN AND THE DISCOVERY AND DEVELOPMENT OF MOTILINOMIMETICS

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SUMMARY

Motilin is a peptide which stimulates gastro-intestinal motor activity. Motilin has been mostly studied in relation to the migrating motor complex, and is thought to be involved in the regulation of this pattern. Motilin also increases pressure in the lower oesophageal sphincter, accelerates gastric emptying, induces gallbladder contraction and increases colon motility. Smooth muscle motilin receptors have been characterized pharmacologically in several species and organ-specific subtypes may exist. The existence of neuronal motilin receptors can be deduced from *in vivo* data. They probably resemble the smooth muscle receptors, and may be even closer related to the recently discovered central motilin receptors.

Erythromycin has been shown to be a motilin agonist. The development of motilin antagonists has removed all doubt in this regard. The successful application of erythromycin in patients with gastroparesis has stimulated the development of motilinomimetics, a new class of prokinetic drugs. This class encompasses motilides, derived from macrolides, and motilin analogues. Several motilides have already been proposed as well as two motilin analogues, and affinity for the motilin receptor has been a useful screening tool in their development. At the same time these studies have led to an understanding of the structure-activity relation of motilin, and to the development of antagonists. The motilin receptor may also be used to develop antibiotics with reduced gastro-intestinal side-effects.

One may safely predict that soon motilinomimetics will be available for the treatment of hypomotility conditions. This will again increase our understanding of the physiological role of motilin and of the regulation of gastro-intestinal motility.

INTRODUCTION

Two decades have passed since the discovery of motilin (*Brown* et al., 1972), and until a few years ago this peptide was reduced to the status of an orphan peptide: abandoned by its discoverer, who saw more future in GIP, it attracted the attention of only a few "motilinophiles". The picture changed when the hypothesis that erythromycin was a motilin agonist (*Peeters* et al., 1989), found a successful application in patients with diabetic gastroparesis

	1	6	11	16	21
pig, man	FVPIF	TYGEL	QRMQE	KERNK	GQ
dog	FVPIF	THSEL	QKIRE	KERNK	GQ
cat	FVPIF	THSEL	QRIRE	KERNK	GQ
rabbit	FVPIF	TYSEL	QRMQE	RERNR	GQ
chicken	FVPFF	TQSDI	QKMQE	KERNK	GQ

Figure 1: Amino acid sequence of porcine motilin, and substitutions found in other species.

(*Janssens* et al., 1990). Presentation of these data at the meeting of the American Gastroenterological Association in Washington in 1989 proved to be so stimulating, that at the next meeting in San Antonio in 1990 a whole symposium could be devoted to erythromycin's prokinetic effects. Since then interest continues to grow, as the program of the present Symposium again demonstrates. This paper is not an extensive overview, but a brief and broad summary with a perspective to the past as well as to the future.

MOTILIN

Motilin is a 22-amino-acid polypeptide, first isolated from the duodenal mucosa of the pig by J.C.Brown in 1972 (*Brown* et al., 1972). It was named motilin because of its ability to induce motor activity in the gastro-intestinal tract. Motilin has also been isolated from a few other species. Known sequences are shown in Figure 1.

Motilin has especially been studied in relation to the migrating motor complex (MMC). This is a motility pattern which is observed in the fasted state in man and in several other species and which consists of three phases. In phase 1 motor activity is absent. It is followed by phase 2 when contractions occur irregularly. During phase 3 contractions occur at their maximum frequency. This phase lasts only for some minutes, ends abruptly and may be followed by a brief period of declining irregular activity, phase 4. The migrating motor complex progresses distally from the lower oesophageal sphincter to the terminal ileum. In man this takes about 90 min, and this is also the time interval separating the occurrence of the same phase of the MMC at one particular location. It has been proposed that the MMC functions as the "intestinal housekeeper" keeping the gastro-intestinal tract free of residual food, desquamated cells and intestinal secretions, which would otherwise accumulate during fasting. The MMC may also limit bacterial growth in the proximal small intestine. Indeed bacterial overgrowth is associated with the absence of MMC activity (*Vantrappen* et al., 1977).

Administration of motilin induces phase 3 of the MMC, but the peptide has also other effects. In pharmacological doses, motilin increases pressure in the lower oesophageal sphincter, accelerates gastric emptying, induces gallbladder contractions and increases colonic motility (*Vantrappen* and *Peeters*, 1989). The physiological role of motilin is still debated, but the peptide is considered to be involved in the regulation of the migrating motor complex. The main arguments for this hypothesis are 1) the temporal relationship

between the occurrence of motilin plasma peaks and the occurrence of phase 3 activity in the gastroduodenal area; 2) the induction of phase 3 activity by the administration of motilin in doses which increase plasma levels to the same extent as the endogenous rise; 3) the inhibition of phase 3 activity in the gastroduodenal area by immunoneutralisation of motilin.

Motilin's mechanism of action has not been fully elucidated. *In vitro* it induces contractions in preparations from man, rabbit, cat and chicken which are mediated via smooth muscle receptors (*Strunz* et al., 1976; *Adachi* et al., 1981; *Depoortere* et al., 1993; *de Clercq* et al., 1995). Just as motilins from different species show amino acid substitutions, the smooth muscle receptors of the different species are not identical. This can be deduced from the differences in potency and in binding affinity (*Peeters*, 1993a). Besides these species differences, evidence has also been presented for organ-specific receptor subtypes in the rabbit. However the putative p- and d-receptors of, respectively, the pyloric region and the duodenum, are both smooth muscle receptors (*Peeters* et al., 1994a).

In vitro preparations from dog, rat and guinea pig do not respond to motilin, although the dog has been the model of choice to study *in vivo* effects. *In vivo* however, motilin's effect is neurally mediated, even in species in which a direct smooth muscle effect has been shown *in vitro* (man, rabbit). Motilin appears to stimulate the release of excitatory neurotransmitters via cholinergic, opioid and serotonergic pathways, but may also inhibit the release of inhibitory substances such as VIP and NO. This will not be discussed in detail, but an important finding in this respect is the motilin-evoked release of acetylcholine (*Kitazawa* et al., 1993). The *in vivo* data therefore indicate that besides the smooth muscle motilin receptors mentioned above, and characterized fairly well *in vitro*, there also exist neuronal receptors. Nevertheless all these receptors seem to be closely related, as *in vitro* potencies (reflecting the muscular receptor) correlate well with *in vivo* potencies (reflecting the neuronal receptor) (*Peeters* and *Depoortere*, 1994) . It seems likely that the neural motilin receptors are present in low density in the gastro-intestinal tract, and difficult to demonstrate or characterize in binding studies. This would certainly be the case if these neuronal receptors have a much lower affinity as was suggested by *Kitazawa* et al. (1993). Recently motilin receptors have been discovered in the brain (*Depoortere* et al., 1995). Besides the fact that this opens a new area of investigations, and points to other as yet unknown functions of motilin, one may hope that these central motilin receptors have the same characteristics as the neuronal receptors of the gastro-intestinal tract. This would much facilitate their characterization.

The lack of motilin antagonists has seriously hampered the study of motilin's mechanism of action and physiological role. Recently the first, although weak, motilin antagonist, ANQ-11125 i.e. [Phe^{3}, Leu^{13}] porcine motilin (1-14), was announced (*Peeters* et al., 1994b). Soon thereafter the extended form of this compound OHM-11526 i.e. [Phe^{3}, Leu^{13}] porcine motilin (1-22), was shown to have a slightly higher affinity than motilin itself (*Peeters* et al., 1993), but unfortunately it behaved as a weak agonist *in vivo* (*Matsuo* et al., 1994). The antagonist properties of both these compounds are due to the substitution of residue 3, proline, by phenylalanine. Very recently, it has been claimed that GM-109, a short cyclic peptide with structure Phe-cyclo[LysTyr(3-tBu)-βAla], is

a motilin antagonist, devoid of agonist activity *in vivo* (*Takanashi* et al., 1995). It will be noted that GM-109 has a tyrosine in the position corresponding to the phenylalanine residue in OHM-11526. It may be hoped that these compounds, and derivatives that will certainly be derived from them, will soon help to unravel the physiology of motilin. As will be mentioned below, they have already been helpful to elucidate the mechanism of action of erythromycin derivatives.

DISCOVERY OF THE EFFECT OF ERYTHROMYCIN ON GASTRO-INTESTINAL MOTILITY

The gastro-intestinal side-effects of erythromycin have been known for long, but the mechanisms involved in the induction of nausea, vomiting, cramping, upper abdominal pain and diarrhoea, although rather common, have received little attention. They were vaguely ascribed to changes in intestinal flora, caused by the antibiotic properties of the compound.

In 1984 two groups described, independently and almost simultaneously, that erythromycin stimulated small intestinal contractile activity in the dog. *Zara* et al., (1985) suggested that "part of the macrolide structure might have a direct effect on smooth muscle". *Itoh* et al. (1984) noted that erythromycin mimicked the effect of motilin, and because erythromycin caused a motilin release, they proposed that it acted through motilin.

We explored the possibility that erythromycin acted directly upon a motilin receptor, and showed that erythromycin displaced motilin bound to its receptor, and also mimicked motilin's effect on the rabbit small intestine *in vitro*. We therefore proposed that erythromycin was a motilin agonist (*Peeters* et al., 1989).

Out of the list of effects induced by motilin, acceleration of gastric emptying appears to be the most interesting one from a therapeutic point of view. Our group therefore studied the effect of erythromycin on gastric emptying in patients with diabetic gastroparesis. The results were clear-cut: erythromycin normalized the prolonged gastric emptying times for both liquids and solids in these patients (*Janssens* et al., 1990). As was mentioned in the Introduction, these findings largely stimulated interest in this area of research. The development of more powerful erythromycin derivatives, which lack antibiotic properties, suggests that a new class of powerful prokinetics has been discovered (*Itoh* and *Omura*, 1987).

MACROLIDES, MOTILIDES AND MOTILINOMIMETICS

Erythromycin was discovered in 1953, and is one of a group of substances produced by various species of Streptomyces, microorganisms found in soil. Because these substances all contain a large lactone ring, they are called macrolides. They are divided into 14-membered and 16-membered macrolides depending upon the number of atoms present in the ring structure. Erythromycin, produced by *Streptomyces erythreus*, has a 14-membered ring, while spiramycin, produced by *Streptomyces ambofaciens* is an example of a substance with a 16-membered ring.

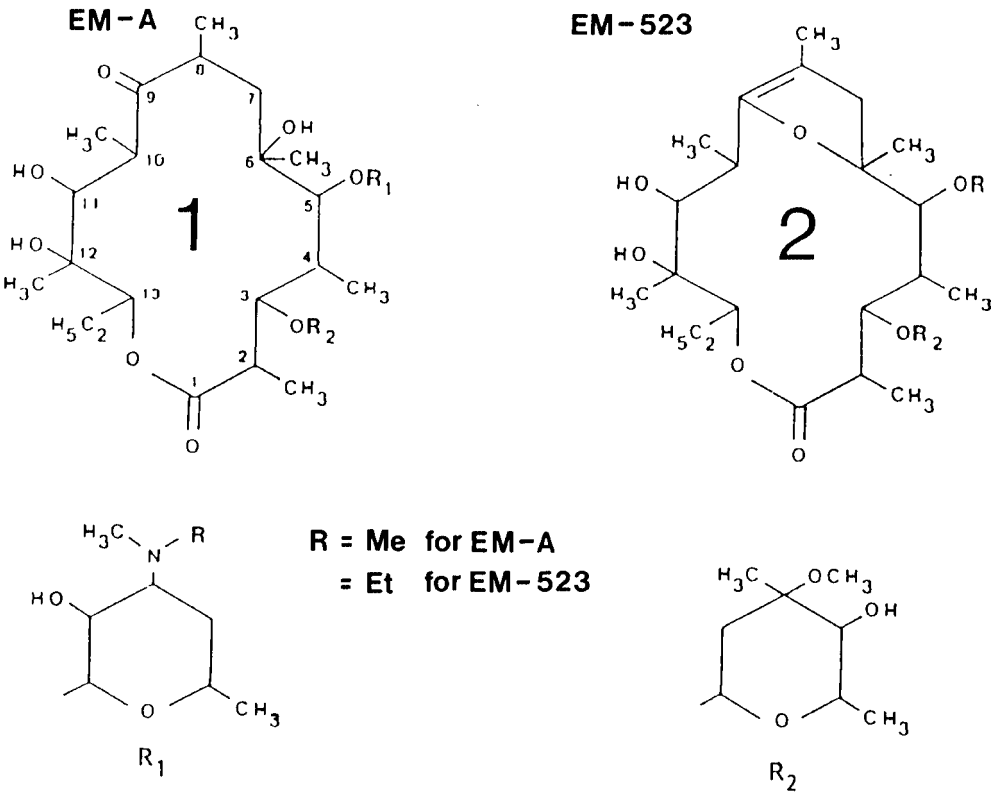

Figure 2: Structure of erythromycin A and of EM-523.

During fermentation, macrolides are produced as a mixture of closely related substances. Thus 3 erythromycin's, designated A, B and C, are produced with A being the major compound. The structure of erythromycin A is shown in Figure 2. As can be seen from this figure, sugars are attached to the ring. In erythromycin A, cladinose is attached in position 3, desosamine in position 5. Erythromycin B differs only from erythromycin A by the absence of the hydroxyl group in position 12, while erythromycin C has in addition a modification of the cladinose sugar.

Attempts have been made to improve the antibacterial properties and the stability of erythromycin (it is destroyed by the acidity of the gastric juice). In 1987 *Omura* et al. (1987) reported that a group of derivatives of erythromycin A lacked antibacterial properties, but had much improved potency in stimulating gastro-intestinal motor activity. *Itoh* and *Omura* (1987) proposed the name motilides for all macrolides with a) a direct contractile effect *in vitro* on rabbit duodenal segments; b) the capacity to induce *in vivo* phase 3 activity in dogs. Although the name motilides may suggest an interaction at the level of the motilin receptor, such an interaction was not being considered and the name mainly referred to the motility effects which did resemble those of motilin.

Our proposal that erythromycin was a motilin agonist (*Peeters* et al., 1989) was met with scepticism, mostly because structurally erythromycin and motilin seem to have little in common. Admittedly the first evidence was incomplete, but it has since then been

Table 1: Motilinomimetics in development.

Compound	Company	Structural features	pKd	Reference
EM-523	Takeda, Japan	14; enol	8.40	Depoortere et al., 1990
EM-574	Takeda, Japan	14; enol	7.94	Satoh et al., 1994
A-81229	Abbott, USA	14; enol	8.14	Nellans et al., 1994
GM-611	Chugai, Japan	14; enol	8.38	Takanashi et al., 1994
LY-274301	Lilly, USA	12; enol	ND	Greenwood et al., 1994
KC-11458	Solvay, Germany	12; enol	ND	Eeckhout et al., 1994
KW-5139	Kyowa, Japan	[Leu13]-po-motilin	9.18	Hanyu et al., 1993
OHM-11638	Ohmeda, USA	motilin fragment analogue	8.94	Macielag et al., 1995

strengthened by other findings. These arguments (for a review see *Peeters*, 1993b) remained indirect, but as mentioned above, motilin antagonists have been discovered recently and they allowed for the unequivocal demonstration that erythromycin is a motilin agonist (*Peeters* et al., 1994b).

Not only erythromycin is a motilin agonist. As expected, many derivatives have the same ability. The substances for which the name motilides was introduced, were mostly derived from 8,9-anhydroerythromycin A 6,9 hemiketal, which is obtained by mild acid treatment of erythromycin A. These conditions favour the addition of the hydroxyl in position 6 to the carbonyl in position 9, followed by dehydratation. The resulting double bond between carbons 8 and 9, and the oxygen bridging carbons 6 and 9, correspond to an enol ether. Introduction of this enol ether configuration in erythromycin's macrolide ring greatly enhances its motility effects. Derivative EM-523 [de(N-methyl)-N-ethyl-8,9anhydroerythromycin A 6,9-hemiketal] synthesized by Dr. Omura from the Kitasato Institute (Tokyo, Japan), is such a compound (Figure 2). We have shown that it is a motilin agonist and that *in vitro* it is about 1000 times more potent than EM-A (*Depoortere* et al., 1990). Other modifications of the ring structure tested so far reduce the potency. Also all macrolides with a 16-numbered ring do *not* interact with the motilin receptor. Structure-activity studies have further revealed that the sugars attached to the macrolide ring are also part of the pharmacophore. Especially modifications of the amino-N of the desosamine sugar may change the potency (*Depoortere* et al., 1989).

At this point we would like to introduce the term motilinomimetics for all compounds able to interact with the motilin receptor, because the term motilides may appear to be too narrow. Indeed, in developing such drugs there is an obvious alternative to the exploration of macrolide derivatives, namely motilin itself. Motilinomimetics therefore encompasses at least two types of drugs: motilin analogues and motilides.

l. Motilin analogues

In order to design a drug starting from motilin itself, a detailed knowledge of the structure-activity relationship is required. Exploration of the affinity for the motilin receptor and of the potency *in vitro* of N-and C-terminal fragments of motilin, and of analogues of the 1-14 fragment in which the residues 1 till 11 where systematically replaced by either alanine or their D-isomer (except residue 8, glycine, which was replaced by D-alanine), led to the conclusion that motilin's pharmacophore resides in the

N-terminal end and involves especially residues 1 (PHE), 4 (ILE) and 7 (TYR) (*Macielag* et al., 1992; *Peeters* et al., 1992).

Motilin has a short half-life, and shorter fragments will most likely also be rapidly metabolized. Very recently the first data on a stabilized motilin analogue, OHM-11638, have been presented (*Macielag* et al., 1995). This compound is an analogue of the (1-14) fragment of porcine motilin stabilized by methylation of the N-terminal amino group, by the introduction of D-Arg in position 12 and of leucine instead of methionine in position 13. Also, the last residue, 14, is lysine instead of glutamine. The affinity for the motilin receptor and the potency *in vitro* of OHM-11638 are comparable to motilin (Table 1). *In vivo* it induces phase 3 activity and accelerates gastric emptying in the conscious dog.

A method has also been developed to synthesize large amounts of porcine motilin by genetic engineering. Only methionine, residue 13, was replaced by leucine because this methionine is easily oxidized and the oxidation product seems to have a reduced biological activity. Encouraging reports on the use of this peptide, KW-5139, have begun to appear in the literature (*Hanyu* et al., 1993). Both OHM-11638 and KW-5139 may prove to be useful as prokinetic agents, but as they require intravenous administration, their application will be limited to acute care patients.

2. Motilides

The enol derivative EM-523 of erythromycin has already been mentioned, and this compound has been developed by Takeda Chemical Company. *Inatomi* et al. (1989) compared the effect of EM-523 on gastric motor activity in conscious dogs, with the effect of cisapride, trimebutine, metoclopramide and motilin. Although the threshold dose for EM-523 was 100 times higher than for motilin, it was 100 times lower than for cisapride which had the lowest threshold dose of the other prokinetics. These data may encourage work with motilin, but they also show that EM-523 is superior to existing prokinetics. Human studies with EM-523 reported up to this date show promising results, but data on a new derivative, EM-574, were recently made available (*Sakai* et al., 1993), suggesting that Takeda Chemical Company also considers other compounds. EM-574 is quite similar to EM-523, the only difference being the replacement of the ethyl group on the desosamine sugar with an isopropyl group.

Abbott Chemical Company has reported extremely high potencies for a compound with code name A-81229 which is closely related to EM-523. It has the enol ring-structure derived from erythromycin B (hydroxyl group missing in C12), the same modification of the desosamine sugar as in EM-523 and an additional modification in the cladinose (hydroxyl at C-4" removed) (*Lartey* et al., 1992). In several models it was shown to have superior prokinetic properties as cisapride (*Nellans* et al., 1994). Another enol-derivative is GM-611, de(N-methyl)-11-deoxy-12-O-methyl-11-oxo-8,9-anhydroerythromycin A 6,9-hemiketal from Chugai Pharmaceutical Company. The compound is acid-stable and has prokinetic properties when administered orally (*Takanashi* et al., 1994).

LY-267108 a ring-contracted derivative (12-membered), which incorporates the enol-structure and in which the two sugars still occupy analogous positions as in Erythromycin, has been developed by Eli Lilly. It increases LES pressure in cats (*Greenwood* et al., 1994). KC-11458, an erythromycin derivative produced by Kali-Chemie Pharma stimulates gallbladder emptying in dogs

(*Eeckhout* et al., 1994). Finally, FK-507, recently introduced as an immunosuppressant, seems to have motilide properties as well (*Ikoma* et al., 1993). Although a macrolide, its structure is quite different and it seems worthwhile to study the interaction of this compound with the motilin receptor.

As yet the motilin receptor has proved to be a useful tool in predicting the prokinetic potential of motilides. Indeed the potency to displace motilin bound to its receptor (pIC_{50}), correlates very well with the potency to induce contractions in segments of rabbit duodenum (pEC_{50}), and this correlates in turn with the potency *in vivo* in other animal models (*Peeters*, 1993a). With such a tool available, one may safely predict that still more potent compounds will be discovered, and that at least one of them will find clinical application.

DEVELOPMENT OF MACROLIDE ANTIBIOTICS

Antibiotic properties are undesirable in a motilinomimetic. Indeed, long term therapy with low doses of a substance with antibiotic properties involves the risk of disturbing the bacterial flora and of developing resistant bacterial strains. Although low doses of erythromycin have a clear prokinetic effect, physicians have been refrained for these reasons from using the drug to stimulate gut motility. It is an advantage of all the motilides that their increased prokinetic potency is accompanied by a strong reduction of their antibiotic properties.

It has been shown that at high doses, such as those used in antibiotic therapy, erythromycin induces abnormal motility patterns (*Sarna* et al., 1991) and it seems likely that these patterns are responsible for at least some of the adverse side-effects of erythromycin. Consequently, antibiotics with reduced affinity for the motilin receptor, may have less gastro-intestinal side effects, at least if they have comparable pharmacokinetical properties. It is interesting to note in this respect that oleandomycin, a 14-membered macrolide with a reduced affinity for the motilin receptor (*Depoortere* et al., 1989), is less potent in inducing motor activity (*Itoh* et al., 1985). Another erythromycin derivative, clarythromycin, is known to have less side effects, and its affinity for the motilin receptor is reduced (*Nellans* et al., 1991). Midecamycin, which like all 16-membered macrolides lacks affinity for the motilin receptor, does not induce gastro-intestinal motor activity and has no or only weak gastro-intestinal side-effects (*Sifrim* et al., 1992). Therefore determining the affinity for the motilin receptor may be useful to predict the likeness that an antibiotic may show gastro-intestinal side-effects.

CONCLUSION AND PERSPECTIVES

The discovery of the effects of erythromycin on gastro-intestinal motility has opened a new area of research and has led to a renewed interest into the peptide motilin. Although it is clear that erythromycin interacts with the smooth muscle motilin receptor *in vitro*, the *in vivo* effects of the motilides are neurally mediated (*Chaussade* et al., 1994). However as this is also the case for motilin, the neural motilin receptor needs characterisation. The discovery of a motilin receptor in the central nervous system (*Depoortere* et al., 1995) may be

very important in this respect.

Another promising development are the motilin antagonists. These compounds will also be helpful in determining the physiological role of motilin. If only recently almost everyone would agree that motilin was mainly related to events occurring in the fasted state, especially the MMC, then the present findings of the wide spectrum of action of motilin agonists, questions this concept.

Therefore, the research reviewed in this paper will not only lead to a new class of prokinetic drugs, and to an understanding of the interaction with the motilin receptor at the molecular level. At the same new insights into the physiological role of motilin, in the regulation of gastro-intestinal motility, and the relation between both will be gained.

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