Gap junction-modifying antiarrhythmic peptides: therapeutic potential in atrial fibrillation

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Abstract

Atrial fibrillation (AF) is the most commonly occurring sustained arrhythmia in clinical practice. AF is a serious disorder associated with an increased risk of stroke, morbidity and mortality, and the number of patients is estimated to more than double within the next 4 decades. The currently available antiarrhythmic drugs (AADs) have limited efficacy and are associated with serious side effects, of which the potentially lethal ventricular proarrhythmias are one of the major concerns. Thus, there is a large unmet clinical need for effective and safe AADs for the treatment of AF. Gap junction-modifying antiarrhythmic peptides (AAPs) that act by increasing cardiac gap junction intercellular communication represent a new class of AADs. Rotigaptide is a synthetic AAP analogue that prevents metabolic stress-induced atrial conduction velocity (CV) slowing and rapidly reverts established atrial CV slowing in vitro. Rotigaptide has contrasting effects in large animal models of AF. In dog models of atrial and ventricular tachypacing-induced AF, rotigaptide significantly increases atrial CV, but does not inhibit electrically induced AF. However, in dog models of ischemia-related AF and chronic atrial dilatation-induced AF, rotigaptide has significant antiarrhythmic effects. Rotigaptide also has a favorable safety profile. In this paper we will review the experimental and clinical data suggesting a role for this new class of gap junction-modifying AAPs for the treatment of AF.

Introduction

Atrial fibrillation (AF) is the most common sustained arrhythmia in the Western population, affecting almost 10% of individuals aged 75 or more (1). It has been estimated that the prevalence of AF in the United States is 2.3 million and it is expected to more than double in 2050, with 5.6 million patients in the U.S. alone (2). AF is a disorder that is most frequently found in patients with chronic cardiovascular disease (e.g., hypertensive, congestive, ischemic or valvular heart disease). AF is a serious disorder associated with an increased risk of stroke, morbidity and mortality (1, 3, 4).

The current treatment paradigm in AF (5) is based on anticoagulation therapy to reduce the risk of stroke, in addition to direct current cardioversion and ion channel-blocking antiarrhythmic drugs (AADs) for the restoration of sinus rhythm. Following sinus rhythm restoration, subsequent AAD treatment is often needed in order to maintain sinus rhythm. In patients in whom sinus rhythm restoration is not possible (or determined to be nonfeasible) treatment is based on the principle of ventricular rate control to alleviate the symptoms (using either drugs or devices) in combination with anticoagulation therapy. The currently available AADs used in the restoration and maintenance of sinus rhythm are associated with frequent side effects, of which the potentially lethal ventricular proarrhythmias are one of the major concerns (6). Furthermore, they may only be effective in preventing recurrent episodes of AF in around 50% of the patient population (7). Thus, there is a large unmet clinical need for effective and safe AADs for the treatment of AF, and the quest for new therapies that effectively maintain sinus rhythm continues.

Numerous experimental and clinical studies have suggested that cardiac conduction slowing and impaired gap junction intercellular communication (GJIC) are important in the pathogenesis of cardiac arrhythmias, including AF. In recognition of this, several authors have proposed gap junction modulation as a new target in the treatment of AF (8-10).

In this paper we will review the experimental and clinical data suggesting an important role for gap junction dysfunction in AF, and will review the experimental data obtained with a new class of gap junction-modifying antiarrhythmic peptides (AAPs) in animal models of AF.
Mechanism of arrhythmia in AF

The pathogenesis of AF is multifactorial and highly complex, as reviewed recently (11). Several different factors contribute to the complex pathogenesis of AF. Among these are: atrial dilatation (12, 13), atrial fibrosis (14, 15), atrial electrical/ionic remodeling (16), atrial ischemia (17), inflammation (18) and oxidative stress (19).

The mechanism of arrhythmia in AF varies among patients, but generally involves two main processes: 1) enhanced electrical automaticity in one or several rapidly depolarizing foci (frequently located in the pulmonary veins) (20); and 2) reentry of one or more reentry waves (21-23). Enhanced focal automaticity can be suppressed by AADs such as amiodarone (24, 25) or by isolating the areas of automaticity through an invasive procedure such as pulmonary vein ablation. Pulmonary vein ablation is highly effective in patients with paroxysmal AF, but less so in patients with chronic AF (26), suggesting that reentry is the predominant mechanism for arrhythmia in chronic AF.

The requirements for initiating a reentry arrhythmia are several and include: electrophysiological inhomogeneity (i.e., spatial differences in conduction and/or refractoriness), unidirectional conduction block and slow conduction. According to the leading circle model of functional reentry (27), the wavelength (the product of the effective refractory period [ERP] and the conduction velocity [CV]) determines the likelihood of reentry by defining the shortest path length in which a reentry wave can sustain itself (Fig. 1A). Several of the ion channel-blocking drugs currently used in the treatment of AF are blockers of the outward potassium channel. At the cellular level, these blockers prolong the atrial ERP. During AF, a prolongation of the ERP results in an increase in the wavelength of the reentry arrhythmia. If the refractory period is sufficiently prolonged by the drug, the wave-length will increase to the point where the arrhythmia can no longer sustain itself, the arrhythmia will terminate, and sinus rhythm will be restored (Fig. 1B) (28-30).

Looking at the wavelength equation, it could be anticipated that treatment with a drug with the ability to increase the other factor in the equation, i.e., the atrial CV, would also lead to an increase in the wavelength and thereby reduce the likelihood of reentry AF (Fig. 1C). Thus, from a mechanistic point of view, a drug with the ability to increase atrial conduction could be very interesting.

Role of conduction slowing in AF

Conduction slowing is an important factor in the mechanism of cardiac reentry arrhythmias, and numerous experimental and clinical studies suggest that atrial conduction slowing is important in the pathogenesis of AF. Experimentally, it has been shown that dogs with inducible AF following cardiopulmonary bypass had decreased atrial CV at baseline compared to animals in which AF could not be induced (31). Atrial conduction slowing has also been associated with the increased AF inducibility seen in old rats compared to young animals (32). Moreover, in dogs subjected to selective atrial ischemia, atrial CV slowing in the ischemic area and border zone was associated with markedly increased AF inducibility (17). Finally, inflammation-induced atrial conduction inhomogeneities following cardiac surgery have been associated with AF development in dogs (33). Clinically, preoperative atrial conduction slowing was found significantly more often in patients developing postoperative AF than in patients who remained in sinus rhythm after cardiac surgery (34), confirming the experimental finding in dogs (31). Atrial conduction slowing has also been reported in patients with a history of paroxys-
most abundant connexins, whereas Cx45 is detected only at very low levels (43).

The level of GJIC and its contribution to the conduction of cardiac action potential is determined at three levels: 1) by gating of the individual gap junction channels; 2) by the expression level of the connexins; and 3) by the spatial distribution of the gap junctions.

Gap junction conductance at the single channel level is regulated in a highly complex manner by a number of stimuli, as previously reviewed (44). Increased levels of intercellular calcium (45), reduced pH (46) and hypoxia (47) are examples of stimuli that reduce GJIC and may lead to cardiac conduction slowing. Pharmacological uncoupling of atrial GJIC using the nonspecific inhibitor of GJIC heptanol leads to atrial CV slowing and increased AF susceptibility in rats (32) and dogs (48), demonstrating that reduced GJIC can be the underlying cause of AF.

Altered connexin expression has also been associated with atrial conduction slowing and atrial arrhythmogenesis. Thus, homozygous Cx40 knockout mice (having no Cx40 expression in the atrium) have reduced atrial CV and increased susceptibility to inducible atrial tachyarrhythmias compared to wild-type mice (49, 50). Furthermore, in a goat model of AF, the atrial expression of Cx40 becomes significantly reduced with the duration of the arrhythmia (51), and reduced expression of atrial Cx40 protein has also been reported in AF patients (52). However, not all investigators have found connexin expression to be reduced in AF. Thus, atrial levels of Cx40 and Cx43 have been reported to be increased in a dog model of AF (53), as well as in some patients with AF (54).

**Gap junction dysfunction is associated with AF**

Atrial conduction slowing may result from structural changes (38), reduced excitability (39) or impaired GJIC (40, 41). This section will only focus on the role of gap junctions in atrial conduction slowing and atrial arrhythmogenesis.

In the heart, intercellular gap junction channels are responsible for the synchronized spread of the action potential from cardiomyocyte to cardiomyocyte, and the level of GJIC is an important determinant of cardiac conduction (40, 41).

Connexins are the main functional components of the gap junction, as they form intracellular channels that connect the cytoplasmic compartments of neighboring cells (42) (see Fig. 2). Atrial cardiomyocytes are interconnected by channels composed of various types of connexin proteins. In the human atrium, Cx40 and Cx43 are the two most abundant connexins, whereas Cx45 is detected only at very low levels (43).

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![Fig. 2. Schematic drawing of a gap junction with intracellular channels connecting two neighboring cells. Adapted with permission from: http://en.wikipedia.org/wiki/Image:Gap_cell_junction.svg#file.](http://en.wikipedia.org/wiki/Image:Gap_cell_junction.svg#file)
The spatial distribution of connexins and its correlation to AF has also been examined. The majority of studies concern the spatial distribution of Cx40. In dogs with 7-week-old MI, the level of atrial Cx40 was significantly reduced and the distribution was heterogeneous compared to controls (55). Interestingly, this was associated with an increased AF sustainability. In accordance with this, atrial Cx40 distribution was also reported to be inhomogeneous in the goat model of AF (51), and the degree of heterogeneity in Cx40 distribution correlated with the stability of the arrhythmia (56). Moreover, in patients with a history of AF, a 2.7-fold increase in the atrial expression of Cx40 and a spatial redistribution of Cx40 compared to patients in sinus rhythm were reported (57), and in patients undergoing coronary artery bypass surgery (having no prior history of AF), a high level of expression and a heterogeneous distribution of Cx40 protein were associated with an increased risk of developing postoperative AF (58). Altered distribution of Cx43 has been also associated with AF. Thus, abnormal Cx43 distribution has been reported in dogs with pacing-induced AF (53) and in patients with AF (14).

In conclusion, there is evidence supporting that abnormal connexin gating, connexin expression and connexin distribution increase the susceptibility to AF. General conduction slowing or the presence of small islands of intra-atrial conduction block resulting from a decreased gap junction conductance, altered gap junction expression and heterogeneous spatial distribution of gap junctions may provide turning points for the multiple waves, and thereby promote reentry of impulses. It follows that modification of gap junction gating, expression or distribution may be potential new targets in the treatment of atrial reentry arrhythmias.

**Gap junction-modifying AAPs**

The family of AAPs is a new class of investigational drugs targeting gap junctions in the heart, thereby affecting cardiac conduction. The history of their discovery is shown in Figure 3 and will be described below.

In the early 1980s, a Japanese group described a hexapeptide derived from bovine atria that synchronized the beating of cultured chick cardiomyocytes (59, 60). Due to its effect in vitro, it was named antiarrhythmic peptide (AAP). AAP had no effects on classical membrane currents (61) and its mechanism of action remained a mystery for more than a decade. In the early 1990s, a German group led by Stefan Dhein synthesized a number of AAP analogues, among which AAP10 was the most stable and potent (62). The mechanism of action of AAP10 was discovered in 1997, when it was reported to increase GJIC in pairs of ventricular cardiomyocytes (63). Due to their poor stability, AAP and AAP10 were unsuitable as future AADs, but the problem with poor stability was solved when Zealand Pharma synthesized a highly stable AAP analogue, named rotigaptide (formerly known as ZP123). Rotigaptide (Fig. 4) is a rotation-inversion of AAP10 that incorporates unnatural D-amino acids to provide improved proteolytic stability (64). AAP10 and rotigaptide are equipotent in vitro (64, 65), and like AAP10, rotigaptide increases gap junction conductance (66). The chemical name of rotigaptide is: acetyl-D-tyrosyl-D-prolyl-trans-4-hydroxy-D-prolyl-D-alanyl-glycine amide, abbreviated to Ac-D-Tyr-D-Pro-D-Hyp-D-Ala-Gly-NH₂. The molecular formula is C₂₈H₃₉N₇O₉ and the molecular weight is 617.7 g/mol.

In 2002, although the therapeutic potential of the AAPs had already been established with rotigaptide in animal models of ventricular tachycardia, the potential of the AAPs in AF was unknown. There was only one report in the literature on the effect of AAPs on atrial tissue. Thus, AAP was reported to restore the rhythmic movement in isolated atria beating in an arrhythmic fashion due to treatment with high concentrations of acetylcholine and low concentrations of potassium (59). In the period 2002-2006, we conducted a series of in vitro and in vivo phar-
macology studies to determine the therapeutic potential of the class of AAPs in AF. We used AAP10 and rotigaptide for the various proof-of-principle studies. The experimental strategy was first to examine the effects of an AAP analogue on atrial electrophysiology using a simple in vitro model, before moving into pharmacological efficacy studies in more complex in vivo models of AF. In the following sections, the results from our studies will be reviewed and discussed.

Effects of rotigaptide on atrial conduction

We measured the effect on rotigaptide on atrial conduction in an isolated rat atrial strip model. We determined the full concentration-response relationship of rotigaptide under physiological conditions and during metabolic stress. Under physiological conditions, rotigaptide had no effect on rat atrial CV. In contrast, it had significant effects on conduction in the setting of metabolic stress. Thus, in isolated rat atrial strips, rotigaptide maintained normal conduction during a 40-60-min period of metabolic stress (69, 70). Rotigaptide was also able to revert established CV slowing in the isolated rat atrial strip model (70). In a dog model of selective atrial ischemia-induced atrial conduction slowing, rotigaptide had no effect on conduction in the nonischemic zone, whereas it prevented the atrial CV slowing in the ischemic zone during a period of 5 h of ischemia (71), consistent with findings in the rat atrium (69, 70). In addition to the effect on CV, rotigaptide also attenuated the ischemia-induced increase in conduction heterogeneity index, implying that conduction was more uniform after treatment with rotigaptide (71). This is an interesting finding, as spatial differences in conduction are one of the electrophysiological abnormalities that may increase the likelihood of reentry.

Previously, it was reported that rotigaptide (at a concentration of 80 nmol/l) had no effect on ventricular CV in isolated guinea pig hearts under baseline conditions, whereas it significantly attenuated acidosis-induced CV slowing (67). Moreover, in studies isolated papillary muscle, AAP10 had no significant effect on activation time under physiological conditions, whereas during metabolic stress it significantly reduced activation time (72). Furthermore, in recent studies on human primary osteoblasts, rotigaptide was reported to have only minor effects on GJIC under normoxic conditions, whereas it normalized GJIC in the setting of metabolic stress-induced cell-to-cell uncoupling (73). Thus, the demonstration that rotigaptide preferably affects conduction in poorly coupled atrial tissue is in accordance with previous findings with AAP10 and rotigaptide from three different laboratories.

In contrast to these studies are data reported by Guerra et al. (74). In their study in normal control dogs, rotigaptide treatment significantly increased atrial CV. The reason for this discrepancy is unclear, although one possible explanation could be that they used isoflurane to anesthetize the dogs. Isoflurane has been described to induce a modest but significant reduction of gap junction conductance (75). Thus, it is possible that due to a side effect of isoflurane anesthesia, the dogs may have had their gap junctions pharmacologically uncoupled and that this uncoupling was reversed with rotigaptide.

During metabolic stress, the concentration-response relationship of rotigaptide was bell-shaped in the isolated rat atria, with maximal efficacy in the concentration range from 10 to 100 nmol/l (Fig. 5). A bell-shaped dose-response curve for rotigaptide was also reported in an in vivo model of CaCl2-induced cardiac conduction block in mice (64), and in a reporter gene-based gap junction assay (76). Thus, our findings in the rat atrial strip regarding the shape of the concentration-response relationship are in accordance with previous findings.

The in vitro and in vivo studies examining the effects of rotigaptide on ventricular conduction and ventricular arrhythmias all involved healthy animals (i.e., with normal hearts), or tissue from healthy animals in which acidosis, ischemia or metabolic stress was induced (64-67, 77). Metabolic stress and ischemia were essential and highly relevant when examining the potential of this class of compounds in ischemia-induced ventricular tachycardia. However, in order to examine the potential of gap junction-modifying AAPs in AF, other models had to be used taking into account the different pathophysiology in AF compared to ischemia-induced ventricular tachycardia. As AF is most frequently encountered in patients with underlying chronic cardiovascular diseases (e.g., congestive, hypertensive, ischemic and valvular heart disease), models mimicking these underlying diseases had to be used.

To date, the effect of rotigaptide on atrial CV has been evaluated in four different animal models characterized by chronic electrical and/or structural remodeling mimicking various clinical contexts in which AF occurs. We examined the effect of rotigaptide on atrial CV in rabbits with

Fig. 5. Concentration-response relationship of rotigaptide in isolated rat atria. The figure shows the changes in conduction velocity (ΔCV) after 40 min of metabolic stress in vehicle (■) and rotigaptide- treated rat atria (values are expressed as relative to baseline). Values are mean ± SEM, n=4-8/group. Reprinted with permission from Ref. 69.
chronic atrial dilatation (a model of AF in patients with chronic atrial dilatation caused by, e.g., valvular heart disease) using high-resolution optical mapping on isolated Langendorff perfused rabbit hearts (78). In this model, rotigaptide significantly increased atrial CV (Fig. 6).

In a subsequent study (71), multipolar electrode arrays with 240 bipolar electrodes were used to examine the effect of rotigaptide on atrial CV in a dog model of atrial tachypacing (a model mimicking the electrophysiological consequences of AF with shortening of the atrial refractory period being the most prominent feature), and in a dog model of ventricular tachypacing (a model of AF in patients with congestive heart failure). In parallel with these studies, Dr. Olgin’s group examined the effect of rotigaptide in a dog model of mitral regurgitation-induced chronic atrial dilatation and in a dog model of ventricular tachypacing-induced heart failure using multipolar electrode arrays with 512 unipolar electrodes (74). Rotigaptide increased atrial CV in all dog models, with an increase of 13-42% (Table I). Studies in dogs and in dilated rabbit hearts were the first to report the effects of an AAP analogue in the remodeled heart and strongly indicate that the effect of rotigaptide on CV is preserved in the remodeled heart. Moreover, the data are supported by a recent publication demonstrating a significant effect of AAP10 on ventricular conduction and arrhythmogenesis in a chronically remodeled heart (isolated rabbit hearts with healed MI) (79). Interestingly, whereas rotigaptide had no effect on atrial CV in the normal and unremodeled heart under physiological conditions, it increased atrial CV under baseline conditions in all remodeled AF models. The consistent effect of rotigaptide on atrial conduction that was observed in the four very different animal models, using two different recording techniques, suggests that chronic remodeling processes that lead to an increased AF inducibility (whether caused by atrial or ventricular tachypacing or by chronic atrial dilatation) are associated with functional uncoupling of the atrial gap junctions, which is reversible following treatment with rotigaptide.

In chronically dilated rabbit hearts, there was a significant downregulation of both Cx43 and Cx40 expression in chronic atrial dilatation (a model of AF in patients with chronic atrial dilatation caused by, e.g., valvular heart disease). In this animal model, there was a significant downregulation of both Cx43 and Cx40 expression.
slowed atrial conduction (86), and therefore seemed appropriate to examine the effect of rotigaptide. In the isolated, chronically dilated rabbit hearts, atrial reentry arrhythmias were induced in 3 of 7 rotigaptide-treated hearts following rapid pacing compared to 3 of 5 vehicle-treated hearts (78). Thus, rotigaptide had no overall effect on AT inducibility in this model. We used optical mapping to characterize the type of arrhythmias induced. Single-wave reentry arrhythmias were recorded in all episodes of atrial arrhythmias induced (see example in Figure 8), whereas arrhythmias with focal activation were not recorded. Rotigaptide has been reported to exert significant antiarrhythmic effects on ventricular reentry arrhythmias (66), but to lack an effect on focal ventricular arrhythmias (77). Therefore, we would have anticipated that rotigaptide would have reduced atrial tachycardia inducibility as the mechanism of arrhythmias was reentry and there was a significant increase in CV as described above. In contrast, in the isolated rabbit heart, rotigaptide failed to prevent the inducibility of reentry AT. Rotigaptide increased atrial CV in the hearts where AT could be induced, as well as in the noninducible hearts, suggesting that the lack of antiarrhythmic effect of rotigaptide was not due to a lack of effect on atrial CV. However, in the AT-inducible hearts, atrial CV after treatment with rotigaptide was lower than in the noninducible hearts, indicating that the atrial CV simply did not reach a certain threshold to prevent AT in these animals.

Table I: Summary of electrophysiological effects of rotigaptide.

<table>
<thead>
<tr>
<th>Animal model of AF</th>
<th>Maximal effect on atrial CV</th>
<th>Antiarrhythmic effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat model of asphyxia-induced AF (82)</td>
<td>ND</td>
<td>No</td>
</tr>
<tr>
<td>Rabbit chronic atrial dilatation model (78)</td>
<td>+14%</td>
<td>No</td>
</tr>
<tr>
<td>Dog ventricular tachypacing model (71)</td>
<td>+13%</td>
<td>No</td>
</tr>
<tr>
<td>Dog atrial tachypacing model (71)</td>
<td>+23%</td>
<td>No</td>
</tr>
<tr>
<td>Dog chronic atrial dilatation model (74)</td>
<td>+42%</td>
<td>Yes</td>
</tr>
<tr>
<td>Dog atrial ischemia model (71)</td>
<td>+52%</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Fig. 7. Duration of atrial fibrillation(s) in rats before and after treatment with vehicle, AAP10 and rotigaptide; n=5-8 rats/group. Adapted with permission from Figure 2 in Ref. 82.
structural remodeling of the atria, one of the hallmarks being atrial fibrosis. To study the effects of rotigaptide in the structurally remodeled atria, we used a dog model of ventricular tachypacing (89). Rotigaptide had no effect on AF duration or on AF vulnerability, despite a significant effect on atrial conduction in this model (71). Thus, the results from the atrial and ventricular tachypacing models are in accordance with the results from the isolated rabbit heart study in which rotigaptide also increased atrial CV without having an antiarrhythmic effect (78). The lack of effect in the ventricular tachypacing model is also in accordance with findings from Guerra et al. (74), who examined the effect of rotigaptide in a ventricular tachypacing model (Fig. 9).

The reason for the lack of effect of rotigaptide in the atrial and ventricular tachypacing models is unclear. It has been reported by several investigators that the mechanism of AF in the ventricular tachypacing model is focal arrhythmia originating from the pulmonary veins or the atrial wall (90, 91). In the atrial tachypacing model, the mechanism of arrhythmia has been reported in accordance with the data from dogs with atrial ischemia (71). The significant antiarrhythmic effect of rotigaptide was in contrast to the finding in the rabbit model of chronic atrial dilatation. A possible explanation for this discrepancy between the two studies could be that the increase in CV in the dog model was 3-fold larger than in the rabbit model (78) (Table I).

The effect of rotigaptide on arrhythmogenesis was examined in four different dog models of AF (71, 74). The effect of rotigaptide was studied in a dog model of mitral regurgitation-induced chronic atrial dilatation (74). In this model, rotigaptide significantly reduced AF vulnerability at a plasma concentration of 100 nM (Fig. 9). The antiarrhythmic effect was associated with an increase in atrial CV of 42% at plasma concentrations of 100 nM (74). Rotigaptide also decreased the conduction heterogeneity index in dogs with chronic atrial dilatation, in accordance with the data from dogs with atrial ischemia (71). The significant antiarrhythmic effect of rotigaptide was in contrast to the findings from the rabbit model of chronic atrial dilatation. A possible explanation for this discrepancy between the two studies could be that the increase in CV in the dog model was 3-fold larger than in the rabbit model (78) (Table I).

Pertinent to this discussion is the role of gap junction remodeling. Gap junction remodeling resulting from rapid atrial activation during AF is well described in patients with AF and is one of the key pathophysiological factors of sustained AF (11). To study the effect of rotigaptide on the electrically remodeled atria, we used a dog model of atrial tachypacing for 3-6 weeks (71). This model has many similarities to the ionic remodeling seen in patients with AF (87, 88), and in this model rotigaptide had no effect on AF duration nor on AF vulnerability (for discussion see below).

Heart failure is a frequent underlying disease in patients with AF. Heart failure is associated with severe structural remodeling of the atria, one of the hallmarks being atrial fibrosis. To study the effects of rotigaptide in the structurally remodeled atria, we used a dog model of ventricular tachypacing (89). Rotigaptide had no effect on AF duration or on AF vulnerability, despite a significant effect on atrial conduction in this model (71). Thus, the results from the atrial and ventricular tachypacing models are in accordance with the results from the isolated rabbit heart study in which rotigaptide also increased atrial CV without having an antiarrhythmic effect (78). The lack of effect in the ventricular tachypacing model is also in accordance with findings from Guerra et al. (74), who examined the effect of rotigaptide in a ventricular tachypacing model (Fig. 9).

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was used. Moreover, the plasma levels of rotigaptide were in the range of the effective plasma levels reported in the dog VT studies (66, 68). However, we cannot rule out that higher dose levels of rotigaptide would have had an antiarrhythmic effect in the atrial and ventricular tachypaced dogs. Given the bell-shaped dose-response relationship of rotigaptide, we can also not rule out whether a lower dose level of rotigaptide could have been effective in preventing AF. Nevertheless, the fact that we observed a significant antiarrhythmic effect in ischemic and mitral regurgitation dogs at the same dose levels makes it less likely that the use of a suboptimal dose was the reason for the lack of antiarrhythmic effects in the atrial and ventricular tachypaced dogs.

Effects of rotigaptide on atrial refractoriness

Rotigaptide had no atrial refractoriness-prolonging effect in any of the dog models of AF studied (71, 74). This is in accordance with previous reports on the lack of effect of rotigaptide on ventricular repolarization (64, 66, 67). The currently available class III AADs all increase atrial ERP as part of their mechanism of action. The investigational atrial-selective ion channel-blocking drugs also increase the atrial refractory period. The lack of effect of rotigaptide on atrial refractoriness confirms that this compound belongs to a new class of AADs, with a completely different mechanism of action compared to the existing drugs.
Safety pharmacology studies with rotigaptide

A major limitation of the use of the currently available ion channel-blocking AADs is their proarrhythmic potential, a side effect that is related to the effect on ventricular repolarization. In order to examine the proarrhythmic potential of rotigaptide, we performed a series of in vitro studies evaluating its effects on HERG channel activity, as well as its binding specificity against a broad panel of 80 receptors and ion channels. Rotigaptide had no significant HERG channel-blocking activity at concentrations that affected atrial conduction (69). Moreover, rotigaptide showed no or very low affinity for all 80 ion channels and receptors examined (69). This finding confirms the lack of effect of rotigaptide on membrane currents observed in double-cell patch-clamp studies on ventricular cardiomyocytes (66) and the reported lack of effect on action potential shape or duration (64, 66, 67, 77). Moreover, rotigaptide had no effect on triggered activity or delayed afterdepolarizations in vitro (77). Taken together, these data suggest that it is unlikely to have any proarrhythmic potential.

Rotigaptide had no effect on contractility in the isolated rat atra, neither under baseline conditions nor during metabolic stress (69, 70). Furthermore, in the dog AF models, rotigaptide had no effect on systemic hemodynamics. The lack of effect of rotigaptide on cardiac contractility, heart rate and blood pressure confirms previous findings (64, 66, 68) and suggests that rotigaptide is unlikely to have any negative inotropic effects in humans.

In parallel with the in vitro safety pharmacology studies, a series of in vivo safety studies were performed with the purpose of examining the effect of rotigaptide on infarct size after MI. The background for initiating the studies was a number of reports demonstrating that pharmacological uncoupling of GJIC with nonselective compounds significantly reduces infarct size in the setting of an acute MI (94-97). Moreover, mice with heterozygous knockout of Cx43 subjected to coronary artery ligation were reported to develop smaller infarcts compared to wild-type mice (98). Based on these observations, several authors raised concerns that compounds with the ability to increase GJIC could lead to larger infarcts during MI (98, 99).

To investigate the effect of rotigaptide on infarct size, we performed two large in vivo studies. Using a rat model of chronic MI and a dog model of ischemia-reperfusion, we determined that treatment with rotigaptide did not increase infarct size. In fact, rotigaptide treatment was associated with a reduction in infarct size in both studies (68, 100). The conclusion from the two studies is that although treatment with nonselective compounds known to decrease GJIC has been reported to reduce infarct size, this information cannot be extrapolated to the effects of compounds that selectively increase GJIC. Extrapolating to the clinical situation, there are no data suggesting that treatment with rotigaptide in patients with AF could be associated with a risk of increasing infarct size should the patients experience an acute coronary syndrome (ACS). This is very important, as the majority of AF patients have an increased risk of experiencing an ACS due to their underlying heart disease.

The stress dependency of the effect of rotigaptide in the isolated rat atria (69) confirms previous findings with AAP10 and rotigaptide, and the stress dependency concerns the effect on both ventricular conduction and the dispersion of ventricular refractoriness (64, 67, 72). The reason for the stress dependency is unknown, but could be related to the fact that under physiological conditions, gap junction conductance is near maximal, and therefore pharmacological stimulation with AAP compounds cannot increase the GJIC further. The preference for AAP analogues to exert their effect during metabolic stress and ischemia may be an important issue in terms of the safety of this class of compounds. Rotigaptide was recently reported to increase conductance in cells coupled through gap junctions formed by Cx43, whereas it had no effect on cells coupled by gap junctions formed by Cx26 or Cx32 (101). Whether rotigaptide affects GJIC in cells expressing other types of connexin is unknown. However, it is clear that the effect of rotigaptide on GJIC is not restricted to cells of cardiac origin, as rotigaptide also increases GJIC in primary human osteoblasts (73). If the preference of AAP analogues to act on poorly coupled cells applies to all organ systems in which Cx43 is expressed, this may represent a beneficial quality in terms of preventing unwanted side effects on organs that are not exposed to acute metabolic stress.

Mechanism of action of rotigaptide on atrial tissue

The molecular target through which rotigaptide exerts its effect has not yet been discovered. Rotigaptide increases GJIC between ventricular cardiomyocytes (66) and it was recently demonstrated that rotigaptide also increases GJIC in the atrial-derived cell line HL-1 (101). Rotigaptide has no effects on basal membrane currents (66), does not exhibit cardiac sodium channel-blocking effects (67) and has no significant HERG channel-blocking activity (69). Furthermore rotigaptide does not bind to a panel of 80 different ion channels and receptors (69). Thus, it appears reasonable to conclude that rotigaptide selectively increases atrial GJIC and that this effect underlies the effect on atrial conduction reported in vitro and in vivo.

Theoretically, rotigaptide could increase atrial GJIC by affecting connexin gating, expression and distribution. In support of an effect on connexin gating is the finding that rotigaptide increases the activity of protein kinase C (PKC) and increases the phosphorylation of the major cardiac gap junction protein Cx43 (65). Furthermore, rotigaptide site-specifically suppresses dephosphorylation of serine residues in the C-terminal tail of Cx43 in rat hearts subjected to ischemia (102). Phosphorylation of the C-terminal tail of Cx43 is an important regulator of connexin gating (103) and these data suggest that the effect of rotigaptide could be mediated through an effect on connexin gating. However, connexin phosphorylation not only...
affects channel gating, but also the connexin expression and turnover rate, as recently reviewed (104). Thus, it cannot be ruled out that some of the effects of rotigaptide could be mediated through an effect on connexin expression. One group of investigators reported that rotigaptide had no effect on Cx43 expression (101). However, another group reported that rotigaptide increased the expression of Cx43 in cultures of rat neonatal cardiomyocytes (105). The increased Cx43 expression was associated with an increased formation of gap junctions, as determined using fluorescence microscopy. These in vitro data are further supported by the observation that rotigaptide treatment was associated with a significant increase in the number of gap junctions in the ischemic border zone of dogs subjected to ischemia-reperfusion (68). In conclusion, the in vitro and in vivo data suggest that rotigaptide may affect both connexin gating and connexin expression. Whether rotigaptide has any effect on connexin distribution is not yet known.

**Perspectives and future directions**

Clearly, the experimental data support the potential use of rotigaptide and related compounds that stimulate GJIC in ischemia-related AF (e.g., new-onset AF in patients with acute MI and AF in patients with ischemic heart disease). Interestingly, new-onset AF occurs in 7-10% of patients experiencing an acute MI (92, 93) and is an independent predictor of death in these patients. Thus, treatment with a drug that prevents new-onset AF (as rotigaptide did in dogs) could have important clinical implications in terms of improved survival.

The experimental data also support the use of rotigaptide and related compounds in patients with AF caused by atrial dilatation and severe CV slowing (as was the case in the dog model of chronic atrial dilatation) (74). Chronic atrial dilatation is associated with atrial conduction slowing and an increased risk of developing AF (12, 13, 83, 84). Thus, a drug with the ability to increase atrial conduction would be expected to be effective in preventing arrhythmia in patients with atrial dilatation and concomitant atrial conduction slowing.

An intriguing question is whether rotigaptide treatment will have any antiarrhythmic effect in AF patients in which the arrhythmia substrate is not acute ischemia, or in the clinical setting where atrial conduction slowing is not the key pathophysiological mechanism of the arrhythmia. An important and consistent finding was that rotigaptide increased atrial conduction under baseline conditions in the remodeled atra (71, 74, 78), in contrast to the lack of effect of rotigaptide in normal hearts under similar conditions. Whether rotigaptide will have the same preference for acting on the remodeled myocardium in humans with AF or those at risk of developing AF is unclear. If this were the case, we would expect that rotigaptide treatment would increase atrial CV in patients with AF substrates such as congestive, hypertensive and valvular heart disease. Whether an increase in atrial CV in these patients would translate into an antiarrhythmic effect remains to be determined. The preclinical data from the atrial and ventricular tachypaced dogs and from the rabbits with chronic atrial dilatation suggest that a minor increase in conduction is not enough to prevent AF (71, 78). Although the effect of rotigaptide on atrial CV in these studies was not sufficient to prevent the inducibility of AF or reduce AF sustainability, these data do not exclude a potential beneficial effect of rotigaptide in patients with AF related to congestive or hypertensive heart disease. Clinical studies are warranted to examine the acute electrophysiological effects of rotigaptide in different AF patient populations.

As mentioned previously, the wavelength (i.e., the product of the ERP and the CV) determines the likelihood of reentry by defining the shortest path length in which a reentry arrhythmia can sustain itself (27). By increasing CV, rotigaptide increases the wavelength in the setting of reentry arrhythmia according to the leading circle theory. As we did not calculate the wavelength during AF in any of the experimental studies, it is not possible to conclude whether the rotigaptide-mediated increase in CV during steady-state pacing was also accompanied by an increase in the wavelength during AF. Analyzing the effect of rotigaptide on fibrillatory conduction in future studies is warranted and would be expected to provide important information on the effect of rotigaptide on wavelength dynamics.

An interesting question is whether combination treatment with rotigaptide and a class III AAD could result in increased efficacy. By attacking both the CV factor and the refractoriness factor in the wavelength equation, the likelihood of reentry should be reduced compared to single-drug treatment. Moreover, if co-treatment shows additive efficacy, the dose of the class III compound might be able to be reduced, with a reduced risk of side effects. The combined effect of rotigaptide on reentry arrhythmias and the effect of class III compounds on arrhythmias with focal origin would also be expected to lead to improved efficacy compared to single-drug treatment regimens. Whereas the classical class III compounds and the newer atrial-selective class III-like drugs would be expected to act synergistically with rotigaptide, it is more difficult to predict the effect of co-treatment with rotigaptide and class I AADs like flecainide and propafenone, whose main action is to reduce cardiac CV through blockade of the cardiac sodium channel. The reason for this is that the mechanism by which rotigaptide increases conduction is mechanistically different from the mechanism by which the class I compounds reduce CV. Studies examining the efficacy and safety of co-treatment with rotigaptide and the classical AADs are warranted.

Another intriguing question is whether long-term treatment with a gap junction modifier like rotigaptide would have any effect in addition to the acute electrophysiological effects described to date. If gap junction downregulation and remodeling are causally related to the arrhythmia and not just an epiphenomenon, it would be expected that changes in connexin expression could have beneficial effects. In this respect, the observation that rotigaptide increases cardiomyocyte Cx43 expression in vitro (105)
and the number of gap junctions in the ischemic border zone in dogs with acute MI (68) makes it tempting to speculate that long-term treatment with rotigaptide may affect the underlying AF substrate by increasing connexin expression. Experimental in vivo studies examining the effect of long-term treatment with rotigaptide on connexin expression and connexin remodeling are warranted to further explore the potential effect of the drug.

The effect of rotigaptide on gap junctions formed by Cx40 has not been examined to date. Cx40 is widely distributed in the atria and plays a major role in atrial conduction during both sinus rhythm (49, 50, 106) and AF (107). Cx40 is not present in the rat atrium (108), which suggests that the electrophysiological effects of rotigaptide in the isolated rat atrium are mediated through an effect on Cx43. However, both Cx40 and Cx43 are present in the rabbit (109) and dog atrium (110). Thus, whether the effect of rotigaptide on atrial conduction in the rabbit (78) and dog atrium (71, 74) was mediated through an effect on Cx43 and Cx40 or through an effect on Cx43 alone is unclear. Future studies examining the effect of rotigaptide of GJIC in cells coupled by Cx40 are warranted.

Due to its low oral bioavailability (unpublished data), it will be difficult to develop an oral formulation of rotigaptide for use in the chronic AF indication. Clearly, it is of paramount importance to develop an orally available AAP analogue, as antiarrhythmic treatment with such a drug is expected to be long-lasting, if not chronic, in patients with AF. An orally available AAP analogue could also potentially be used as an alternative to implantable cardioverter defibrillator (ICD) implantation in the preventive treatment of patients with recurrent episodes of VT.

Conclusions

Rotigaptide concentration-dependently prevented conduction slowing and reverted established conduction slowing in the isolated rat atrium. Moreover, rotigaptide treatment was associated with a significant antiarrhythmic effect in a dog model of atrial ischemia-induced AF and in a dog model of chronic atrial dilatation. In animal models of AF and congestive heart failure-related AF, rotigaptide treatment increased atrial CV, but the effect on conduction was not associated with an antiarrhythmic effect.

Taken together, the data reported in this review support the potential use of rotigaptide and related compounds that stimulate GJIC in ischemia-related AF and in patients with severe atrial conduction slowing. Moreover, our data suggest that rotigaptide treatment has the potential to increase atrial conduction in hearts with different underlying AF substrates. Although the effects on atrial conduction in the experimental models were not sufficient to prevent the inducibility of AF or reduce AF sustainability, our data do not exclude a potential beneficial effect of rotigaptide in patients with these AF substrates. Future studies examining the effect of rotigaptide in patients with AF will hopefully increase our understanding of the therapeutic potential of this new class of antiarrhythmic compounds.

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