MiniReview

Pharmacological Modulation of Gap Junction Function with the Novel Compound Rotigaptide: A Promising New Principle for Prevention of Arrhythmias

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Abstract: Existing anti-arrhythmic therapy is hampered by lack of efficacy and unacceptable side effects. Thus, ventricular tachycardia and fibrillation remains the strongest predictor of in-hospital mortality in patients with myocardial infarction. In atrial fibrillation, rhythm control with conventional ion channel blockers provide no therapeutic benefit relative to rate control. Several lines of research indicate that impaired gap junctional cell-to-cell coupling between neighbouring cardiomyocytes is critical for the development of cardiac re-entry arrhythmias. Rotigaptide is the first drug that has been developed to prevent arrhythmias by re-establishing gap junctional intercellular communication. During conditions with acute cardiac ischaemia, rotigaptide effectively prevents induction of both ventricular and atrial tachyarrhythmia. Moreover, rotigaptide effectively prevents ischaemia reperfusion arrhythmias. At the cellular level, rotigaptide inhibits ischaemia-induced dephosphorylation of Ser297 and Ser368, which is considered important for the gating of connexin43 gap junction channels. No drug-related toxicity has been demonstrated at plasma concentrations 77,000 times above therapeutic concentrations. In rats and dogs, rotigaptide reduces infarct size following myocardial infarction. A series of phase I trials has been completed in which rotigaptide has been administered intravenously to ~200 healthy persons. No drug-related side effects have been demonstrated in healthy human beings. Clinical safety, tolerability and efficacy in patients with heart disease are being evaluated in ongoing clinical trials. Rotigaptide represents a pioneering pharmacological principle with a highly favourable preclinical and clinical safety profile, which makes this molecule a promising drug candidate for the prevention of cardiac arrhythmias.

Unmet medical need in the treatment of ventricular tachycardia and atrial fibrillation

Sudden cardiac death is one of the leading causes of death in the Western world accounting for more than 250,000 lives yearly in the USA alone [1]. In the majority of cases, sudden cardiac death is caused by ventricular tachycardia (VT) and ventricular fibrillation (VF). The underlying disease responsible for the vast majority of these arrhythmias is ischaemic heart disease leading to myocardial infarction (MI) [2]. The attempts to prevent ventricular tachyarrhythmias by pharmacological intervention in patients recovering from MI have been disappointing. In this patient population, most anti-arrhythmic drugs seem to be without effect on overall mortality [3] or even increase mortality [3–5]. During the past decade, treatment of MI patients has improved substantially through the wide introduction of angioplasty, stenting and new methods for preventing thrombus formation, but despite these important advances the presence of sustained VT/VF remains one of the strongest predictors of in-hospital mortality [6]. Moreover, in a recent prospective trial on 45,852 patients with MI, it was demonstrated that while inhibition of platelet aggregation with clopidogrel reduced the risk of reinfarction and 28-day mortality, the number of deaths related to VT/VF (about 25%) were unchanged [7]. These recent trials confirm that effective prevention of VT/VF could have a large impact on mortality in this patient population.

Whereas ventricular tachyarrhythmias accounts for the majority of the cardiovascular mortality, atrial fibrillation (AF) is the most common sustained arrhythmia in the Western patient population, affecting almost 10% of persons aged 75 or more [8]. It has been estimated that the prevalence of AF in the USA is 2.3 million [9] and it is expected to be more than doubled in 2050 with 5.6 m patients in the USA alone [9]. AF is most frequently found in patients with chronic cardiovascular disease (e.g. hypertensive, congestive, ischaemic and valvar hear heart disease) and is a serious disorder associated with an increased risk of stroke, morbidity and mortality [10,11,8]. The currently available anti-arrhythmic drugs used for restoration and maintenance of sinus rhythm may only be effective in preventing recurrent episodes of AF in around 50% of the patient population [12]. Furthermore,
these agents have well-defined side-effect profiles including potentially lethal ventricular pro-arrhythmias that are also encountered in the treatment of VT [13]. Thus, there is still a large unmet clinical need for efficacious and safe anti-arrhythmic drugs for the treatment of VT and AF.

The pro-arrhythmic potential of the currently available anti-arrhythmic drugs for the treatment of VT and AF seems to be linked to their ion channel-modulating effects. Therefore, the traditional target of modulation, that is, cardiac ion channels might be abandoned in favour of new approaches to develop effective anti-arrhythmic agents.

In the last decade, numerous experimental and clinical studies have suggested that cardiac conduction slowing and impaired gap junction intercellular communication (GJIC) may be important in the pathogenesis of cardiac arrhythmogenesis. Therefore, modulation of gap junction conduction could be a very interesting target in the development of new and safe anti-arrhythmic drugs [14–17].

The cardiac gap junction

Gap junction plaques are specialized regions of the cell membrane with clusters of hundreds to thousands of densely packed gap junction channels that directly connect the cytoplasmic compartment of two neighbouring cells [18]. Gap junction channels are composed of two hemichannels (connexons) provided by each of two neighbouring cells. Each connexon consists of six proteins called connexins. The connexins are a large family of proteins (i.e. in the human genome 20 connexin genes have been identified and in the mouse genome 19 have been identified) [19]. All connexins share the basic structure of four transmembrane domains, two extracellular loops and a cytoplasmic loop. There is a high degree of conservation of the extracellular domains, two extracellular loops and a cytoplasmic loop. Connexins share the basic structure of four transmembrane domains, two extracellular loops and a cytoplasmic loop. The distribution of the different types of connexins varies throughout the heart. The three major connexin isoforms of the heart are: connexin43 (Cx43), connexin40 (Cx40) and connexin45 (Cx45). Cx43 is the predominant type in the ventricles and is also widely expressed in the atria but only sparsely expressed in the conduction system. Cx43 is localized to special regions of the cell membrane called intercalated discs. Cx40 is the most abundant isoform in the conduction system but is also widely expressed in the atria (except rat), whereas Cx45 mainly is found in the conduction system [20].

Gap junction channels can switch between an open and a closed state by a twisting motion. In the open state, ions and small molecules up to ~1 kDa can pass through the pore [21]. Thus, intercellular coupling concerns both electrical conductance and metabolic coupling, both of which depend on the number of expressed channels, the single channel conductance or permeability and the open probability of a single channel. A number of different stimuli such as hypoxia [22] and reduced pH [23,24] lead to closure of gap junction channels and reduced conductance.

Gap junction plaques can be strictly side-to-side or end-to-end or a combination of both, allowing activation wave-fronts to move in both the longitudinal and the transverse direction [25]. Due to the elongated shape of the cells, wave-fronts moving in the transverse direction must traverse more intercellular junctions. Gap junction resistance is considerably higher than cytoplasmic resistance and, therefore, propagation in the transverse direction is slower than in the longitudinal direction. This difference in conduction velocity (CV) gives rise to the phenomenon of anisotropy.

The anti-arrhythmic peptides

In the early 1980s, a Japanese group described a hexapeptide derived from bovine atria that synchronized the beating of cultured chick cardiomyocytes [26,27]. Due to its effect in vitro, it was named the anti-arrhythmic peptide (AAP). In the early 1990s, a German group led by Stefan Dhein synthesized a number of AAP analogues, among which AAP10 was the most potent [28]. AAP10 was reported to increase GJIC in pairs of cardiomyocytes [29]. However, due to its poor stability, AAP10 was unsuitable as a future anti-arrhythmic drug. Therefore, we developed a new AAP analogue called rotigaptide (formerly known as ZP123), which is a rotation-inversion of AAP10 that incorporates unnatural D-amino acids to provide improved proteolytic stability [30] (fig. 1).

Cellular mechanism of action of rotigaptide

Rotigaptide increases gap junction intercellular communication. To confirm that rotigaptide, like AAP10, increases GJIC, we conducted a series of double cell patch clamp studies on isolated guinea pig cardiomyocytes that demonstrated that rotigaptide increases GJIC in adult cardiomyocytes without affecting membrane conductance [31] (fig. 2).

To address the question of cellular selectivity of rotigaptide, Clarke et al. investigated the effects of rotigaptide on cell-to-cell dye transfer following microinjection of small fluorescent dyes in three cell model systems: rat neonatal cardiac myocytes, HL-1 cells (atrial cardiomyocyte derived cell line) and in HeLa cells transfected and selected to express different connexins [32]. Rotigaptide had no effect on dye transfer in HeLa cells expressing Cx32- or Cx26-green fluorescent protein (GFP) fusion proteins or wild-type Cx26. In contrast, HeLa cells expressing Cx43-GFP exposed to 50 nM rotigaptide for 5 hr showed a 40% increase in GJIC and similar findings were observed in neonatal rat cardiomyocytes and atrial HL-1 cells, where Cx43 is the dominant connexin. Therefore, the longer carboxyl tail of Cx43 may be important for the signalling of rotigaptide. The carboxyl tail of Cx43 is subject to posttranslational modification especially phosphorylation, the extent of which is dynamically regulated and appears to influence channel permeability.
To evaluate whether rotigaptide also affects GJIC by increasing the number of gap junction channels, a few studies have examined the effects of rotigaptide on Cx43 expression. Using a detection antibody against Ser368 Clarke et al. found that rotigaptide had no effect on expression of Cx43 in rat neonatal cardiac myocytes and in Cx43-GFP labelled HeLa cells [32]. In contrast, Stahlhut et al. reported that rotigaptide produced a dose-dependent increase in Cx43 protein expression in cultured rat neonatal cardiomyocytes and immunocytochemical studies suggested increased Cx43 immunoreactivity was particularly increased at the cell-to-cell interface [33]. The most compelling evidence to support
that rotigaptide may affect the amount of gap junctions between cells was found in the ultrastructural study by Hennan et al. [35]. Ultrastructural analysis of infarcted hearts from dogs treated with vehicle or rotigaptide was examined by transmission electron microscopy. The degree of damage was comparable among the vehicle and the rotigaptide-treated animals. In the infarct border zone and in the myocardium outside of the risk zone, the frequency of gap junctions was similar among treatment groups but in the non-infarcted myocardium within the risk zone, rotigaptide administration appeared to increase the presence of gap junctions [34,35].

Rotigaptide prevents dephosphorylation of Cx43 during ischaemia.

The cellular target molecule for rotigaptide and the other AAPs is still unknown; however, the molecular mechanism responsible for the effect of the AAPs has been investigated in a few studies. The effect of AAP10 on GJIC can be antagonized by the specific protein kinase C (PKC) inhibitor bisindolylmaleimide and by inhibition of PKC-\(\alpha\), suggesting a role for PKC-\(\alpha\) in AAP10 signalling [36]. The finding that AAP10 significantly activates PKC in HeLa Cx43 cells, and that PKC-\(\alpha\) inhibitors can block this activation supports this [36]. Furthermore, AAP10 enhances phosphorylation of Cx43 [37]. When G-protein coupling was inhibited with GDP-\(\beta\)S, the effect of AAP10 on phosphorylation was abolished, suggesting that AAP10 binds to a G-protein-dependent membrane receptor and enhances Cx43-phosphorylation by activation of PKC [37].

Using the same HeLa-Cx43 cell line, Dhein et al. demonstrated that both natural AAP, AAP10 and rotigaptide stimulates PKC activity and that this effect was completely blocked by the selective PKC-\(\alpha\) inhibitor CGP54345 [38].

To further evaluate the hypothesis that rotigaptide regulates GJIC by interacting with phosphorylation at certain phosphorylation sites of the Cx43 C-terminal, Axelsen et al. studied changes in Cx43 phosphorylation during global ischaemia in the absence and presence of rotigaptide [39]. Phosphorylation analysis was performed on Cx43 purified from isolated perfused rat hearts using matrix-assisted laser desorption/ionisation mass spectrometry and liquid chromatography electrospray ionization tandem mass spectrometry. Thirteen different serine phosphorylation sites were identified in Cx43 during non-ischaemic conditions, three of which had not previously been described. Within the first 7 min. of ischaemia, Ser306 became fully dephosphorylated whereas Ser330 became phosphorylated. Between 15 and 30 min. of ischaemia, the critical time interval where gap junction uncoupling occurs, Ser297 and Ser368 also became fully dephosphorylated. During the same time period, all untreated hearts developed asystole. Treatment with rotigaptide significantly increased the time to ischaemia-induced asystole and suppressed dephosphorylation of Ser297 and Ser368 at 30 min. of ischaemia (fig. 3). Axelsen et al. confirmed that Ser368 was phosphorylated by PKC-\(\alpha\)

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**Fig. 3.** Serine phosphorylation of Cx43 during ischaemia. This figure summarizes the findings regarding serine phosphorylation of Cx43 during ischaemia. Serine sites marked in red have been identified as phosphorylated at either non-ischaemic or ischaemic conditions. As indicated by the red arrow, Ser330 became phosphorylated within the first 7 min. of ischaemia; whereas Ser306 became dephosphorylated, as indicated by the blue arrow. Ser297 and Ser368 became dephosphorylated between 15 and 30 min. of ischaemia and Ser330 become dephosphorylated between 30 and 45 min. of ischaemia. The green stars at Ser297 and Ser368 indicate that phosphorylation of these sites was preserved for 30 min. of ischaemia by pre-treatment with rotigaptide. Reproduced with permission from Elsevier; Axelsen et al. [39].
and PKC-ε, while PKA, PKC-α, PKC-ε, PKG, adenosine monophosphate-dependent kinase, p38 mitogen-activated protein kinase and Ca²⁺/calmodulin-activated protein kinase II were all unable to phosphorylate synthetic peptides containing the newly described rotigaptide-sensitive phosphorylation site, Ser297 [39]. These results suggest that phosphorylation of Ser297 and Ser368 may be involved in functional gating of Cx43 during ischaemia and may be possible downstream targets for rotigaptide signalling.

The use of rotigaptide in the treatment of ventricular tachycardia

Mechanism of VT following acute MI.

Very little is known about the mechanism responsible for ventricular tachyarrhythmias during acute MI in human beings. Approximately 28% of the patients suffering from acute MI die before getting to the hospital. Another 26% experience so called ‘silent ischaemia’ that goes unattended and may only be discovered post-mortem or at a subsequent episode of MI [40]. The rest of the patients are admitted to the hospital where the initial goal is to stabilize the patient and minimize the myocardial damage (i.e. re-open the occluded artery by coronary angioplasty or thrombolysis). Thus, current knowledge on the complex mechanisms involved in the genesis of these life-threatening arrhythmias during the acute phase of MI rests on animal experiments. These experiments have shown that re-entry is the main mechanism involved in the initiation and maintenance of ischaemia-induced VT [41–43] and the degeneration of VT to VF causing sudden death [42–45]. However, focal mechanisms such as triggered activity due to injury currents flowing between the ischaemic and the normal area probably also play a role [42,43,45–47]. In addition, re-entrant and focal mechanisms can contribute in the same run of VT (i.e. initiation occurring by one mechanism and maintenance occurring by another).

Clinical and experimental mapping studies of arrhythmias in the subacute and chronic phase of MI have shown that re-entry, also in these stages, is the main mechanism involved in the initiation and maintenance of VT [48–52]. Re-entry occurs when the propagating impulse does not die out after activation of the heart, but persists to re-excite the heart after the end of the refractory period. Mapping studies of activation wavefronts have shown that re-entry can take different forms (leading circle, figure-of-eight, multiple wavelet re-entry, micro re-entry) depending on the properties of the tissue it arises in [50,51,53]. The prerequisites for re-entry are unidirectional conduction block and slow conduction. Two major principles for the formation of unidirectional block have been described. One suggests that dispersion of action potential duration/refractoriness is the primary cause of unidirectional conduction block [54], whereas the other emphasizes the anisotropic properties of cardiac tissue as the underlying cause of block [55]. In the acute and subacute stage of MI, both principles are likely to participate concomitantly; however, as membrane properties tend to normalize during infarct healing, anisotropic re-entry is the most likely mechanism involved in the chronic stage of MI.

Role of gap junctions in ventricular conduction and VT.

An increased understanding of the important role of gap junctions in ventricular conduction has been provided by the development of genetically engineered Cx43 mice. Mice homozygous for a targeted deletion of the Cx43 gene die at birth from cardiac and pulmonary malformations [56]. Heterozygous mice survive, but have significantly slowed conduction compared to wild-type mice [57] and a higher incidence of spontaneous VT during regional ischaemia [58]. Moreover, mice with a heart-specific knockout of Cx43 die by 2 months of age from sudden cardiac death caused by spontaneous ventricular arrhythmias [59]. A reduced expression and an altered distribution pattern of Cx43 has also been described in hearts from patients with different cardiac diseases associated with an increased risk of arrhythmias such as congestive heart failure, dilated cardiomyopathy, inflammatory cardiomyopathy, hibernation, cardiac hypertrophy and Chagas’ disease [18,60–66]. Moreover, in a study in which induction of VT was attempted by programmed stimulation in dogs with 4-day-old healing infarcts [67], sustained VT could only be induced in the dogs with a marked disruption of gap junction distribution with abnormal Cx43 labelling along the lateral surface of the cells throughout the full thickness of the surviving layer of the border zone at sites correlating with the location of re-entrant VT circuits [67]. However, in the dogs in which sustained VT could not be induced the abnormalities did not extend through the full thickness of the surviving layer [67]. Altogether, these data clearly prove an important role for Cx43 in the maintenance of normal ventricular conductance and demonstrate that down-regulation and heterogeneous expression of Cx43 may lead to ventricular arrhythmias.

Rotigaptide prevents increased dispersion of action potential duration during ischaemia and acidosis.

Dispersion of action potential duration (APD) and effective refractory period arises due to the heterogeneous effects of ischaemia on ion channel and gap junction function throughout the ischaemic area. The dispersion is especially pronounced in the border zone separating the ischaemic area from the normal myocardium [68,69]. Uncoupling of GJIC further augments the dispersion. Normally, in well-coupled cells, APD dispersion is smoothened by the electrotonic forces exerted by neighbouring cells (i.e. a cell with a long APD next to a cell with a short will tend to prolong the duration of the short and vice versa). However, uncoupling will prevent this smoothening and contribute to an unmasking of dispersion of action potential duration and refractory period [70]. Thus, beat-to-beat APD variability is larger in isolated ventricular myocytes than in electrically coupled myocytes [71]. Dhein et al. have shown that increasing concentrations of the selective gap junction uncoupler palmitoleic acid dose-dependently increased the dispersion
of activation-recovery interval without influencing the mean duration measured at 256 electrodes in the isolated rabbit heart [72].

As increased dispersion of action potential duration/refractoriness may lead to the formation of unidirectional conduction block and re-entrant VT, it is important to prevent increased dispersion. Several studies have showed that rotigaptide indeed is capable of preventing increased dispersion. Thus, in isolated rabbit hearts, rotigaptide (0.1 nM) significantly reduced the dispersion of activation-recovery intervals measured by 256 epicardial electrodes [38] and reduced the increased dispersion of action potential duration in hearts subjected to hypokalaemic ischaemia [30]. A similar effect was seen in isolated guinea pig hearts subjected to acidosis [73].

Rotigaptide prevents slow conduction during ischaemia and acidosis.

During the acute and subacute phase of ischaemia, conduction velocity is severely depressed both by an effect on membrane excitability and on GJIC. This combined effect of inhibition of fast inwards sodium channels and cellular uncoupling at gap junctions leads to a significantly slowed conduction velocity in both the longitudinal and transverse direction [74]. Slow conduction facilitates the occurrence of re-entry because it allows time for the tissue in the re-entrant circuit to recover its excitability, thereby allowing the re-entrant wavefront to continue propagation.

As mentioned above, double cell patch clamp experiments have shown that rotigaptide increases GJIC in guinea pig cardiomyocytes [31]. Therefore, rotigaptide may also be

![Fig. 4. Rotigaptide inhibits acidosis-induced conduction slowing. Contours illustrate propagation from a point stimulus proceeding in an elliptical pattern. Action potential upstrokes (inset) recorded from five sites (C) along the transverse axis of propagation illustrate relative conduction delay between recording sites. In the absence of rotigaptide (A and B), acidosis produced significant slowing of conduction. In contrast, pre-treatment with rotigaptide (C and D) preserved conduction during acidosis. ZP123 attenuates acidosis-induced conduction velocity (CV) slowing while producing no change in pattern of propagation. \( \theta_L \) indicates longitudinal CV; \( \theta_T \) indicates transverse CV. Reproduced with permission from Lippincott, Williams and Wilkins; Eloff et al. [73]](image-url)
expected to increase conduction velocity (CV) in the intact myocardium. This hypothesis was tested by Eloff et al. who used high-resolution optical mapping to measure CV from ventricular epicardium of Langendorff-perfused guinea pig hearts at baseline (pH 7.4) and during 45 min. perfusion with acidic (pH 6.0) Tyrode’s solution with and without 80 nM rotigaptide. They showed that acidosis significantly slowed conduction both transverse and longitudinal to cardiac fibres [73]. Importantly, rotigaptide inhibited conduction slowing in both directions and maintained a normal anisotropic ratio (fig. 4). As expected, the effect was most pronounced in the transverse direction where conduction slowing was reduced by ~60%. Rotigaptide’s peak effect was achieved after 16 min. of acidosis, consistent with inhibition of uncoupling [73]. The effect of rotigaptide on CV was confirmed in isolated guinea pig hearts subjected to global low-flow ischaemia where rotigaptide prevented the ischaemia-induced slowing of CV [75].

Rotigaptide prevents re-entrant but not focal VT during acute MI.

Based on the fact that rotigaptide prevents dispersion of action potential duration and conduction slowing, it may be hypothesized that rotigaptide would also prevent the occurrence of re-entrant VT. This hypothesis was tested by three-dimensional mapping in the open-chest dog following ligation of the left anterior descending coronary artery (LAD) [31]. This technique provides a unique opportunity to distinguish between arrhythmias of focal and re-entrant origin. Briefly, 23 multipolar plunge-needle electrodes were inserted into the myocardium in the risk zone of LAD ligation. Each electrode gave rise to up to six bipolar electrograms providing recordings throughout the myocardial wall. VT was induced by programmed electrical stimulation 1 to 4 hr after LAD ligation. The stimulation protocol was very aggressive using up to four premature stimuli, resulting in a very high degree of reproducibility of re-entrant VT in the saline-treated dogs (~90%). However, all three doses of rotigaptide (giving rise to plasma concentration of approximately 1, 8 and 70 nM) convincingly prevented the induction of re-entrant VT [31]. The preventive effect of rotigaptide on re-entrant VT was closely correlated to reversal of functional, unidirectional conduction block (fig. 5A and B). At the highest concentration, rotigaptide reversed conduction block in eight out of the nine experiments in which rotigaptide had a preventive effect on re-entrant VT. Conversely, rotigaptide failed to reverse the conduction block in all four experiments without effect on re-entrant VT. By preventing the formation of unidirectional block and conduction slowing during ischaemia rotigaptide eliminates the underlying basis for development of re-entry.

The importance of GJIC for the development of focal arrhythmias is less well described; however, computer simulation studies have suggested that GJIC can influence the automaticity and the propagation of focal arrhythmias [76]. Thus, at low values of GJIC as is seen during ischaemia, islands of surviving cells may be spontaneously active; however, the impulse cannot propagate due to the low level of conductance. At high levels of conductance, the surrounding cells suppress automaticity, whereas at intermediate coupling there is a window in which automaticity is not sufficiently suppressed and the coupling is sufficient for propagation of the impulse leading to focal VT. In light of this, it may be hypothesized that rotigaptide may either prevent focal VT.

Fig. 5. Rotigaptide prevents conduction block and the induction of ventricular tachycardia (VT) in dogs. Ventricular pacing in the same dog before treatment with rotigaptide (A) and after rotigaptide administration (B). Before rotigaptide treatment three premature stimuli (asterisks) produced conduction delay and unidirectional conduction block leading to re-entrant VT whereas conduction block and VT could not be induced after rotigaptide administration even after adding one more premature stimuli. From top to bottom are shown recordings from lead II electrocardiogram and epicardial electrograms (E-) near the pacing site (-P) and west (-W), northwest (-NW), southwest (-SW), surrounding the centre (-C), south (-S), east (-E) and underlying subendocardium (SE-). Reproduced with permission from Blackwell Publishing: Xing et al. [31].
by suppressing automaticity or facilitate focal VT by increasing coupling enough for the impulse to propagate but not enough to suppress the activity. The effect of rotigaptide on focal arrhythmias was evaluated in a subsequent study in the open-chest dog [77]. The study design and dosing regimen was similar to the re-entry VT study with the exception that epicardial collaterals were not ligated and pacing was performed from the endocardium in order to increase the frequency of focal arrhythmias. In this study, rotigaptide failed to prevent focal arrhythmias (11 out of 13 had focal VT/VF following rotigaptide treatment, whereas 13 out of 14 had focal VT/VF after saline treatment) suggesting that GJIC plays a minor role, if any, for the development of focal arrhythmias. Although rotigaptide cannot suppress focal activity, it may still prevent the transition from a benign non-sustained focal VT into a malignant sustained re-entrant VT/VF as it is well known that arrhythmias can be initiated by one mechanism and maintained by one or more mechanisms [42,45].

Rotigaptide prevents reperfusion arrhythmias.

First line therapy for patients with acute MI is re-opening of the occluded artery by coronary angioplasty or thrombolysis. Although the benefits of this procedure clearly outweigh the disadvantages, re-opening of the occluded artery may lead to the formation of reperfusion arrhythmias. This form of arrhythmia is often associated with multiple re-entrant VT circuits possibly caused by the marked action potential shortening in the reperfused area giving rise to increased dispersion of action potential duration [78]. Recently, the effect of rotigaptide on reperfusion arrhythmias was tested in open-chest dogs subjected to 1 hr of ligation of the left circumflex coronary artery followed by 4 hr of reperfusion [35,79]. Rotigaptide or saline was given 10 min. prior to reperfusion and premature ventricular complexes and VT was counted for 2-min. periods every 5 min. during the first hour of reperfusion. The two highest doses of rotigaptide significantly suppressed the occurrence of premature ventricular complexes and VT (fig. 6A and B) with a maximal reduction in the highest group of approximately 95% [35,79].

The use of rotigaptide in the treatment of atrial fibrillation

Mechanism of arrhythmia in AF.

The pathogenesis of AF is multifactorial and highly complex. Important factors in the pathogenesis of AF include, but are not limited to: atrial dilatation [80,81], atrial fibrosis [82,83], atrial electrical/ionic remodelling [84,85], atrial ischaemia [86], inflammation [87] and oxidative stress [88]. For a detailed description of the complex pathogenesis of AF, please refer to one of many reviews on the topic that has been published recently [89,90].

AF can be caused by re-entry [91–93], enhanced focal automaticity [94] or a combination of both. Whereas the re-entry mechanism is well documented in patients with chronic AF, the role of enhanced focal automaticity in this patient population is less clear. The fact that pulmonary vein ablation is significantly more efficient in patients with paroxysmal AF than in patients with chronic AF [95] suggests that the focal mechanism is not as important in chronic AF as is the case in paroxysmal AF.

Role of gap junctions in atrial conduction and AF.

Atrial conduction slowing (e.g. as caused by changes in connexin gating, connexin expression and connexin distribution) has been associated with an increased AF susceptibility in numerous experimental studies [86,96–98] and in clinical studies [99–101]. Pharmacological uncoupling of atrial GJIC using the non-specific inhibitor of GJIC heptanol...
is associated with atrial CV slowing and increased AF susceptibility in rats [97] and dogs [102]. Moreover, homozygous Cx40 knock-out mice (having no Cx40 expression in the atrium) have reduced atrial CV and increased susceptibility to inducible AF [103–105]. Abnormal atrial connexin expression has also been reported in a goat model of AF [17] and in human beings with AF [82,106–108]. It is believed that generalized atrial conduction slowing or the presence of small islands of intra-atrial conduction block as a result of decreased gap junction conductance or altered gap junction expression may provide turning points for the multiple waves and thereby promote re-entry of impulses. It follows that modification of atrial gap junction gating, expression or distribution may be potential new targets in the treatment of atrial re-entry arrhythmias.

Rotigaptide increases atrial conduction velocity in vitro.

In an isolated rat atrial strip model, rotigaptide had no effect on atrial conduction during physiological conditions, whereas in the presence of acute metabolic stress (hypoxia + glucose deprivation) rotigaptide concentration dependently prevented stress-induced atrial conduction slowing [109]. This interesting finding that rotigaptide has a preference to act on metabolically stressed atrial tissue is in accordance with the effect of rotigaptide on ventricular conduction [73], confirms findings with AAP10 on isolated papillary muscle [110], and confirms recent studies on human osteoblasts [111].

In the isolated rat atria, the concentration-response relationship of rotigaptide was bell shaped with maximal efficacy in the concentration range from 10–100 nmol/l. A bell-shaped dose-response relationship of rotigaptide and AAP10 was also reported in an in vivo model of CaCl2-induced cardiac conduction block in mice [30] and in a reporter gene-based gap junction assay [112]. The reason for the bell-shaped dose-response relationship of rotigaptide and AAP10 is presently unknown.

In addition to the preventive effect on atrial CV slowing, rotigaptide was also able to revert established atrial CV slowing in the isolated rat atrial strip model [113].

In optical mapping studies on Langendorff-perfused hearts from rabbits with chronic atrial dilatation (mimicking AF in patients with chronic atrial dilatation caused by e.g. valvular heart diseases), rotigaptide treatment significantly increased atrial conduction; however, this effect was not associated with an anti-arrhythmic effect [114]. Importantly, in the chronically dilated rabbit hearts, there was a significant down-regulation of both Cx43 and Cx40 expression. Thus, the effect of rotigaptide on CV may, in this study, be caused either by a direct effect of rotigaptide on single channel conductance or by an effect on connexin expression as suggested by the findings of Stahlhut et al. [33].

Effects of rotigaptide in dog models of AF.

The effect of rotigaptide has been studied in four different dog models of AF, characterized by chronic electrical and/ or structural atrial remodelling mimicking various clinical contexts in which AF occurs.

In an atrial tachypacing model in dogs mimicking the electrophysiological consequences of AF (with the shortening of the atrial effective refractory period being the most prominent feature), rotigaptide increased atrial conduction without having any anti-arrhythmic effect [115]. In a dog ventricular tachypacing model mimicking AF in patients with congestive heart failure, rotigaptide also increased atrial conduction without having any anti-arrhythmic effect [115]. In a dog model of chronic mitral regurgitation-induced atrial dilatation, rotigaptide significantly increased atrial CV. However, in contrast to the atrial and ventricular tachypacing models, this effect on atrial conduction was associated with a significant anti-arrhythmic effect at the 100 nM plasma level [116]. One of the reasons for the discrepancy in efficacy between the models may be magnitude of effect on atrial CV. Thus, in the atrial dilatation model, atrial CV increased by 42% compared to the 5–10% increase in the atrial and ventricular tachypacing models. Finally, in a dog model of selective atrial ischaemia (induced by ligation of a branch from the right coronary artery perfusing the right atrial free wall), rotigaptide treatment was associated with a significant anti-arrhythmic effect as the ischaemia-induced increase in AF duration was completely prevented (fig. 7) [115]. The effect of rotigaptide in this model mimicking new onset AF in the setting of an acute MI was associated with a prevention of atrial conduction slowing in the ischaemic zone. The
significant effect in this model is in accordance with the significant effects of rotigaptide on ischaemia-induced ventricular arrhythmias. Taken together, the experimental data support the potential use of rotigaptide and related compounds that stimulates GJIC in ischaemia-related AF (new onset AF in patients with acute MI, and AF in patients with ischaemic heart disease). Moreover, data suggest that in hearts with different underlying AF substrates, rotigaptide treatment has the potential to increase atrial conduction. Although the effects on atrial conduction in the experimental models were not sufficient to prevent the inducibility of AF or reduce AF sustainability, the 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 the knowledge and our understanding of the therapeutic potential of this new class of anti-arrhythmic compounds.

Pharmacokinetic profile of rotigaptide

The intravenous pharmacokinetics of rotigaptide have been studied extensively in experimental animals and in man. Liquid chromatography/tandem mass spectrometry (LC/MS/MS) methods have been developed and validated for the accurate quantitation of rotigaptide in biological fluids [30]. Based on single intravenous bolus dosages, the pharmacokinetics of rotigaptide in mice, rats and dogs is characterized by moderate total body clearance (CLr) of 0.226 to 0.981 l/hr/kg, a low steady-state volume of distribution (Vdss) of 0.175 to 0.306 l/kg, and a short apparent half-life (t1/2) of 0.29 to 0.9 hr [35] and unpublished data. After repeated intravenous bolus injection or continuous intravenous infusion in rats and dogs, exposure to rotigaptide increased with increasing dosages in an apparently dose-proportional manner.

No metabolites of rotigaptide have been observed in liver microsomes or hepatocytes from mouse, rat, dog or man (unpublished data). In addition, rotigaptide failed to demonstrate inhibition of cytochrome P450 isozymes, suggesting that metabolic drug–drug interactions with concomitant agents that are substrates for these isozymes are unlikely (unpublished data). In animal studies, ~93% of the hexapeptide is excreted in the urine as whole peptide (unpublished data) that further supports the lack of metabolism observed in in vitro models [30].

Safety issues regarding the clinical use of rotigaptide

Rotigaptide has no haemodynamic or electrophysiological side effects. Rotigaptide is a gap junction modifier that is devoid of classical anti-arrhythmic ion channel activity. However, it has been studied extensively for pro-arrhythmic and haemodynamic side effects in order to demonstrate its potential advantages over existing therapies. Unlike existing class I and III anti-arrhythmics, rotigaptide (bolus intravenously, 10 mg/kg) has no effect on arterial blood pressure (systolic, diastolic and mean), heart rate and lead II electrocardiogram in dogs instrumented with telemetry recording systems [35]. There is no evidence of abnormal atrial or ventricular arrhythmia, QT prolongation or morphological changes in any of the electrocardiograms after administration of rotigaptide. PR, QRS and QT values for animals given rotigaptide at 10 mg/kg are comparable to the values observed after the administration of vehicle-control [35]. Plasma concentrations of rotigaptide in this canine cardiovascular safety study were as high as 47,674 ± 1344 ng/ml that is approximately 77,267 times the minimum efficacious concentration observed in the dog open-chest VT model [31]. The electrophysiological activity of rotigaptide was also studied in rabbit Langendorff models [30,38]. Action potential duration and effective refractory period were not changed in the rabbit [30,38] or guinea pig [73] heart after treatment with rotigaptide. In the guinea pig heart, resting membrane potential and action potential amplitude were unaffected by the administration of rotigaptide [73]. Rotigaptide also failed to block the human ether-à-go-go related gene (hERG) channel in an in vitro patch clamp study, further demonstrating the compounds low propensity to prolong the QT interval (unpublished data). Overall, rotigaptide appears to have a preclinical cardiovascular safety profile that will produce less side effects and haemodynamic compromise than current therapy.

Rotigaptide causes a slight reduction of infarct size.

Gap junction uncoupling is a well-known substrate of arrhythmogenesis. However, the role of uncoupling in infarction is poorly understood. Treatment with non-selective compounds that reduce gap junction intercellular communication, such as heptanol, is associated with reduced propagation of hypercontracture and cell death [117,118]. Until recently, investigations into the cardioprotective effect of increasing gap junction communication during ischaemia/reperfusion injury were limited by an absence of compounds that specifically increase GJIC. Rotigaptide has demonstrated a significant reduction in infarct size in a canine ischaemia/reperfusion model (fig. 8) and in a rat-chronic ischaemia infarct model [119]. These data are supported by previous studies demonstrating an important role for GJIC in the transfer of cardioprotective factors during ischaemic preconditioning [120]. The exact mechanism of the cardioprotective effects of rotigaptide remains unknown; however, these results are compatible with the hypothesis that cardiac gap junctions may provide metabolic support from cell to cell during conditions with impaired supply of oxygen and energy (i.e. ‘the kiss of life hypothesis’). Other mechanisms of cardioprotection including altered intracellular calcium dynamics and ATP preservation could also be responsible for the decrease in infarct size observed with rotigaptide. Despite the need for additional studies to better understand the cardioprotection pathways induced by rotigaptide, the reduction in infarction may represent a beneficial quality of a compound being developed for prevention of ventricular arrhythmias associated with acute MI.
ROTIGAPTIDE: A NEW PRINCIPLE IN ARRHYTHMIA PREVENTION

Food and Drug Administration as part of an investigational anti-arrhythmic therapy. These data were presented to the drug with much improved safety margins compared to existing intravenous bolus micronucleus assay (unpublished data). Rotigaptide was also determined to be non-genotoxic in a bacterial reverse mutation assay, chromosome aberration assay and mouse compound-related effects (unpublished data). Rotigaptide for 14 days in dogs were well tolerated and produced no mutation for 5–14 days in rats and dogs. Dosages of rotigaptide were initiated in June 2005 and are currently ongoing. The purpose of these studies was to determine the safety, tolerability and pharmacokinetics of rotigaptide after single intravenous bolus and continuous intravenous infusion in healthy volunteers. Similar to the pharmacokinetic profile observed in non-clinical studies, intravenous bolus injections of rotigaptide in human beings had a disposition with a low CLT of 133 ml/min., low Vdss of 21.4 l and t1/2 of 2.7 hr (121). AUCss was consistent with predicted levels and increased in a dose-proportional manner after continuous intravenous infusion in healthy volunteers [121,122]. Rotigaptide appeared to be safe and well tolerated at the doses tested. Sixty-one per cent to eighty-four per cent of the injected rotigaptide was excreted unchanged in the urine and no metabolites were apparent in the plasma [121,122]. Because rotigaptide is excreted predominately through the kidney, there is a potential for reduced clearance and increased half-life of the drug in patients with renal impairment. Clinical studies are now under way to determine the impact of renal impairment on rotigaptide elimination. Because future clinical development of rotigaptide will require co-administration to patients taking digoxin, an open-label, non-randomized, three-period study was initiated. No pharmacokinetic or pharmacodynamic interaction between rotigaptide and digoxin was observed in this study, thus both drugs can be co-administered safely [123].

Clinical trials

Two phase 1, single centre, double-blind, randomized, placebo-controlled studies of rotigaptide were completed in March 2005. The purpose of these studies was to determine the safety, tolerability and pharmacokinetics of rotigaptide after single intravenous bolus and continuous intravenous infusion in healthy volunteers. Similar to the pharmacokinetic profile observed in non-clinical studies, intravenous bolus injections of rotigaptide in human beings had a disposition with a low CLT of 133 ml/min., low Vdss of 21.4 l and t1/2 of 2.7 hr (121). AUCss was consistent with predicted levels and increased in a dose-proportional manner after continuous intravenous infusion in healthy volunteers [121,122]. Rotigaptide appeared to be safe and well tolerated at the doses tested. Sixty-one per cent to eighty-four per cent of the injected rotigaptide was excreted unchanged in the urine and no metabolites were apparent in the plasma [121,122]. Because rotigaptide is excreted predominantly through the kidney, there is a potential for reduced clearance and increased half-life of the drug in patients with renal impairment. Clinical studies are now under way to determine the impact of renal impairment on rotigaptide elimination. Because future clinical development of rotigaptide will require co-administration to patients taking digoxin, an open-label, non-randomized, three-period study was initiated. No pharmacokinetic or pharmacodynamic interaction between rotigaptide and digoxin was observed in this study, thus both drugs can be co-administered safely [123].

Phase 2 studies investigating the safety and tolerability of rotigaptide in patients who have acute coronary syndrome [unstable angina, ST-segment elevation myocardial infarction (STEMI) or myocardial infarction without ST-elevation] were initiated in June 2005 and are currently ongoing. The compound has been developed to prevent life-threatening re-entry VT/VF, but for ethical reasons, the first safety and tolerability evaluation in MI patients is being conducted in patients with non-lethal ventricular arrhythmias (e.g. pre-ventricular beats and non-sustained VT).

Conclusion and perspectives

Despite major advances in angioplasty and antithrombotic treatment of MI patients, VT/VF remains the strongest predictor of mortality and it accounts for about 25% of the mortality observed during the first 28 days following an MI. In addition, VT/VF complicates patient recovery and adds to the cost of caring for MI patients. Therefore, strategies designed to avoid the development of VT/VF in MI patients are highly warranted.
Table 1. Rotigaptide fact sheet.

<table>
<thead>
<tr>
<th>Key findings</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular structure</td>
<td>Stable hexapeptide (Ac-D-Tyr-D-Pro-D-Hyp-Gly-D-Ala-Gly-NH₂)</td>
</tr>
<tr>
<td></td>
<td>In vitro half-life in human plasma: 14 days</td>
</tr>
<tr>
<td></td>
<td>Highly water soluble</td>
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</tbody>
</table>

**Pharmacology**

- **Cellular level:** Increases Cx43 gap junction intercellular communication in absence of changes in membrane currents
- **Mice:** Prevents ouabain and CaCl₂-induced arrhythmias
- **Rats:** Prevents re-entry ventricular tachycardia (VT)/ventricular fibrillation (VF) arrhythmias but not focal VT/VF during myocardial ischaemia
- **Dogs:** Prevents VT/VF during ischaemia reperfusion
- **Prevents AF in heart failure dogs with mitral regurgitation**

**Pharmacokinetics**

<table>
<thead>
<tr>
<th></th>
<th>Total body clearance (ml/kg/min.)</th>
<th>Volume of distribution (ml/kg)</th>
<th>Terminal elimination half-life (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>12</td>
<td>190</td>
<td>16</td>
</tr>
<tr>
<td>Dogs</td>
<td>4</td>
<td>237</td>
<td>43</td>
</tr>
<tr>
<td>Humans</td>
<td>2–3</td>
<td>280–340 for 70 kg person</td>
<td>138–168</td>
</tr>
</tbody>
</table>

**Cardiac safety pharmacology**

- **Rats:** No effect on PQ, QRS, QT or other electrocardiogram (ECG) parameters
- **Dogs:** No effect on cardiac or systemic haemodynamic parameters
- **Reduces infarct size**

**Other safety pharmacology**

- No effects on vital organ functions (central nervous system, respiratory, cardiovascular) observed following high intravenous dose administration in rats and dogs

**Toxicology**

- No macroscopic or histological toxicological findings have been observed following high dose intravenous administration in animals:
  - **Mice:** 300 mg/kg single dose
  - **Rats:** 100 mg/kg/day for 14 days
  - **Dogs:** 10 mg/kg/day for 14 days
- The maximally tolerated dose has not been identified.

**Clinical experience in healthy human beings**

- No drug-related adverse effects or drug–drug interactions have been observed in these phase I trials:
  - Single ascending dose (SAD) study:
    - Randomized, double-blind, placebo-controlled, in-patient study
    - Total 79 healthy subjects; 8 subjects/group (6 on rotigaptide, 2 on placebo)
    - Doses: 0.03 to 30 mg (24-hr intravenous infusions); 2 and 3 mg (intravenous bolus)
  - Ascending continuous intravenous infusion dose study:
    - Randomized, double-blind, placebo-controlled, in-patient study
    - Total 32 healthy subjects; 8 subjects/group (6 on rotigaptide, 2 on placebo)
    - Doses: 1, 3, 10 and 20 mg/day (Continuous intravenous infusion for 6 days)
  - Drug interactions with digoxin and atenolol:
    - Open label, non-randomized, in-patient study
    - Total 16 (digoxin) and 14 (atenolol + digoxin) healthy subjects
  - Pharmacokinetics study in patients with renal impairment:
    - Open-label, single-dose, parallel-group, non-randomized study in subjects with renal impairment and in healthy subjects. Total 30 subjects, 6 subjects/group.
  - Pharmacokinetic study on the role of age and gender:
    - To evaluate the pharmacokinetics of rotigaptide in healthy men and women of different ages.
    - Open-label, non-randomized, single-dose, parallel-group study of a 24-hr intravenous dose (10 mg) of rotigaptide in healthy men and women of 3 age groups: young, young-older and older subjects.
    - Total 48 healthy subjects: 8 subjects/group/sex.

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Currently, there is no therapeutic option that is approved to prevent ventricular arrhythmias associated with ischaemic events. Despite some anti-arrhythmic effects, conventional ion blocking anti-arrhythmic compounds also possess pro-arrhythmic properties, which make them unsuitable for use in the prevention of VT/VF in STEMI patients.

Rotigaptide does not modulate ion channels, but exerts its anti-arrhythmic effects through modulation of intercellular communication via gap junctions. In view of this novel mechanism and the very favourable preclinical safety data package collected to date, the risk of pro-arrhythmia with rotigaptide appears to be extremely low. Thus, rotigaptide may be ideally positioned to fill the void for an anti-arrhythmic agent that may be administered safely to STEMI patients to prevent fatal ventricular arrhythmias without the potential for pro-arrhythmia, hypotension or cardiac depression, and organ toxicities.

Furthermore, higher frequency of VT/VF has been observed in patients who died suddenly. Among those who died 30 min. after presenting symptoms, 90% had an episode of ventricular arrhythmia. Considering that 25% of acute MI patients die outside of the hospital and the high risk of VT/VF in this population, a therapy to effectively prevent and treat acute VT/VF in this population may provide greater mortality benefit and be more cost-effective than its use in the in-hospital acute MI population. However, an out-of-hospital study can only be completed after safety and efficacy has been shown in an in-hospital setting.

Recently, the first experimental studies on the use of rotigaptide in the treatment of AF have been published. These data suggest that rotigaptide or an orally available second generation molecule could be useful in the treatment of certain forms of AF [115]. Further studies are needed to determine the target population with AF that could benefit from pharmacological modulation of atrial gap junction function.

In summary, rotigaptide represents a pioneering pharmacological principle with a highly favourable preclinical safety profile, which makes this molecule an attractive drug candidate for the prevention of cardiac arrhythmias. All available data on rotigaptide are summarized in table 1. Clinical safety, tolerability and efficacy in patients with heart disease are being evaluated in ongoing clinical trials.

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228 ANNE LOUISE KJØLBYE ET AL.

MiniReview


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230 ANNE LOUISE KJØLBYE ET AL.


