Connexins and Atrial Fibrillation
Filling in the Gaps

Takeshi Kato, MD, PhD*; Yu-ki Iwasaki, MD, PhD*; Stanley Nattel, MD

Atrial fibrillation (AF) is an extremely common arrhythmia with important consequences and presently suboptimal therapeutic options.1 A great deal of research has been performed to understand the detailed mechanisms of AF, with the hope that a better appreciation of the fundamental determinants of arrhythmogenesis will lead to the development of novel, more successful treatment possibilities.2

One aspect of AF pathophysiology that has elicited great interest has been changes in gap junction/connexin physiology. An important role for altered gap junction function and the potential importance of gap junctions as a therapeutic target for AF were first emphasized by Spach and Starmer over 15 years ago.3 Since that time, our understanding of gap junction physiology, biophysics, and molecular biology has increased enormously,4,5 as have studies on their role in AF pathogenesis and management.2

Gap junctions contain transmembrane ion-channel proteins called connexins (Figure, panel A). Connexons (containing 6 connexin molecules each) in the gap junctions of adjacent cardiomyocytes line up and attach, transferring ions or molecules <1 kDa freely between cells, coupling them electrically (Figure, panel B). Cardiac tissues express a variety of connexins, including connexin (Cx)40, Cx43, Cx45, Cx30.2/31.9, and Cx37,5 with all but Cx37 present in cardiomyocytes and the most important connexins in atrial tissue being Cx40 and Cx43. There is evidence that posttranslational modification, particularly connexin phosphorylation, is important in governing connexin localization and function.6

Given the central importance of connexins in cell-to-cell coupling, changes in connexin expression and distribution would be expected to have profound influences on cardiac conduction,2,5 a key determinant of reentry mechanisms that maintain AF.2 Furthermore, connexins can also affect refractoriness heterogeneity, also an important determinant of reentry,2 because tight cell coupling tends to smooth out variations in action potential duration. Thus, it is logical to consider the possibility that changes in connexins might be important in AF pathophysiology.

An important role for connexins in AF is strongly supported by genetic studies indicating that connexin gene variants are associated with AF,7–10 although some of the results regarding specific gene-variants have been contradictory.8–10 A wide range of studies has investigated changes in connexin expression and distribution in clinical and experimental AF models (Table).11–15 Alterations in both total connexin expression (Figure, panel C) and connexin distribution, particularly redistribution from cell-end gap junctions to lateral margins (Figure, panel D), have been described in AF. However, the results show wide variations, with opposing results even within the same models (Table), causing considerable uncertainty about the nature of connexin changes in AF. Transgenic animal models have the potential to provide clearer insights into the role of connexins in AF pathophysiology by revealing the effects of specifically engineered changes in connexins. Here, too, however, there have been contradictory results, with studies indicating a clear increase36 or no change37 in atrial tachyarrhythmia susceptibility with Cx40 knockout. Although Cx40 loss suppresses atrial conduction in knockout mice,36 studies in myocyte strands suggest that Cx40 loss can actually improve atrial conduction.38 Small-molecule drugs that enhance gap junction conductance have been developed as potential treatments for AF, with results showing improvement in some models (ischemia and mitral valve disease–related AF) but little or no change in other clinically relevant paradigms.39–41 Thus, the role of connexin abnormalities in AF and the potential value of modulating connexin function to treat AF remain quite unclear on the basis of the literature.

In this issue of Circulation, Igarashi et al report the results of a novel approach to controlling connexin expression in a porcine model of AF.42 The authors used an epicardial gene-painting approach to transfer Cx40 or Cx43 via adenoviral vectors to right and left atrial tissues, comparing gene-transferred with sham-operated animals. AF was induced by 2-second bursts of 42-Hz, 7.5-V atrial pacing separated by 2-second intervals to observe rhythm. Tachypaced sham dogs were in continuous AF after 5.8±0.6 days, whereas both Cx40- and Cx43-transferred dogs showed significantly increased probability of sinus rhythm, with no significant efficacy differences between Cx40- and Cx43-treated dogs. Atrial conduction was slowed in tachypaced sham dogs and substantially improved by connexin transfer. Tachypaced sham dogs showed significant decreases in total and phosphorylated Cx43 expression, with no change in Cx40 expression. Cx43 transfer normalized Cx43 expression in tachypaced dogs without affecting Cx40 expression; Cx40

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

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Circulation is available at http://circ.ahajournals.org
DOI: 10.1161/CIRCULATIONAHA.111.075432
transfer modestly increased Cx40 expression (by ~20%) and did not affect Cx43 expression.

The results of Igarashi et al are exciting in showing that enhancing connexin expression can have substantial AF-suppressing activity. They also point to a clear pathophysiological role of connexin downregulation in this AF model. In addition, they suggest that gene therapy approaches to increase connexin function and improve AF-related conduction abnormalities may be useful for treating AF. Furthermore, these results are consistent with another recent publication that showed efficacy of adenoviral-mediated Cx43 gene transfer in suppressing AF in a similar porcine model.20 Taken together, the results of these 2 investigations provide novel and important data about the role of connexins in AF.

The study of Igarashi et al has a number of limitations that must be considered. First, no scrambled connexin gene control group was used to control for adenoviral effects perse, and therefore potential nonspecific effects cannot be excluded. Second, the porcine model of Igarashi et al displayed different properties from other prior animal AF models in which atrial tachypacing was used: Conduction was markedly slowed and refractoriness was unaffected, whereas previous models in which tachypacing was used to induce AF showed striking refractoriness abbreviation with very limited conduction slowing.43 Finally, the results of Cx40 gene transfer are difficult to reconcile with the observed physiology. Tachypaced AF dogs showed no Cx40 down-regulation; Cx40 gene transfer increased Cx40 overall expression by only ~20% without affecting intercalated-disk Cx40 expression and failed to alter strong downregulation of total (by ~60%) and phosphorylated (by ~90%) Cx43. Nevertheless, Cx40 gene transfer substantially improved conduction and suppressed AF; it is difficult to understand how this happened.

Despite rapidly increasing knowledge about basic mechanisms underlying AF,2 many crucial mechanistic elements related to clinical AF management remain unresolved.44 The study of Igarashi et al is an important advance with respect to both pathophysiology and therapeutics. From the mechanistic perspective, it provides some of the most solid evidence to date on the potential participation of connexin dysfunction in AF. However, because the investigators studied a very specific animal model, much more work needs to be done to determine the relevance of their findings to various clinical forms of AF. From the therapeutic point of view, the study points the way to further development of connexin gene

Figure. A, Connexons contain 6 connexins each, arranged in a circle. Connexins in 1 cell connect with connexins in adjacent cells across the gap junction, connecting the cells electrically. B, Schematic of cell-to-cell connections in normal heart. C, Atrial fibrillation (AF) can be associated with a change in the number of connexins between cells. D, AF can also be associated with a change in connexin distribution, particularly lateralization of connexins.
transfer therapy to treat AF. Overall, these results highlight the importance of learning much more about the role of gap junctions and their regulation in AF pathophysiology and management.

Acknowledgments
The authors thank France Thériault for excellent secretarial assistance.

Sources of Funding
This work was supported by the Canadian Institutes of Health Research (MOP 44365), the Quebec Heart and Stroke Foundation, the Fondation Leducq (European–North American Atrial Fibrillation Research Alliance), and the MITACS Network of Centers of Excellence.

Table. Results of Previous Studies on Connexins in AF

<table>
<thead>
<tr>
<th>Species</th>
<th>AF Type/Animal Model</th>
<th>Cx40 Protein</th>
<th>Cx43 protein</th>
<th>Remarks</th>
<th>Author</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog</td>
<td>ATP-induced persistent AF</td>
<td>NA</td>
<td>NA</td>
<td>↑</td>
<td>NA</td>
<td>Ablation suppressed AF, Cx43</td>
</tr>
<tr>
<td>Goat</td>
<td>ATP-induced persistent AF</td>
<td>→</td>
<td>↑</td>
<td>→</td>
<td>NA</td>
<td>Van der Velden</td>
</tr>
<tr>
<td>Goat</td>
<td>ATP-induced persistent AF</td>
<td>↓</td>
<td>↑</td>
<td>→</td>
<td>↑</td>
<td>Van der Velden</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Volume overload 8 wk</td>
<td>↓</td>
<td>NA</td>
<td>↓</td>
<td>NA</td>
<td>Rotigaptide did not prevent AF</td>
</tr>
<tr>
<td>Dog</td>
<td>Sterile pericarditis 4 d</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
<td>↑</td>
<td>Transmural Cx40/ Cx43 gradient</td>
</tr>
<tr>
<td>Mouse</td>
<td>TNF overexpression 8–16 wk</td>
<td>↓</td>
<td>↑</td>
<td>→</td>
<td>NA</td>
<td>Lateralization of Cx43</td>
</tr>
<tr>
<td>Dog</td>
<td>Congestive heart failure 2 wk</td>
<td>→</td>
<td>NA</td>
<td>→</td>
<td>NA</td>
<td>Lateralization of Cx43</td>
</tr>
<tr>
<td>Rat</td>
<td>Autoimmune myocarditis</td>
<td>→</td>
<td>NA</td>
<td>↓</td>
<td>NA</td>
<td>Hayano</td>
</tr>
<tr>
<td>Rat</td>
<td>Aldosterone infusion 8 wk</td>
<td>NA</td>
<td>NA</td>
<td>↓</td>
<td>NA</td>
<td>Reil</td>
</tr>
<tr>
<td>Pig</td>
<td>ATP-induced persistent AF</td>
<td>NA</td>
<td>NA</td>
<td>↓</td>
<td>NA</td>
<td>Cx43 transfer prevented AF</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Thyroxine injection 4 wk</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>Lateralization of Cx43</td>
</tr>
<tr>
<td>Rat</td>
<td>Elevated afterload 20 wk</td>
<td>↓</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Cx40 heterogenous, even in SR group</td>
</tr>
<tr>
<td>Human</td>
<td>Post-operative AF (CAD)</td>
<td>↑</td>
<td>→</td>
<td>→</td>
<td>→</td>
<td>Lateralization of Cx40/Cx43</td>
</tr>
<tr>
<td>Human</td>
<td>Chronic AF &gt;1 y</td>
<td>↑</td>
<td>NA</td>
<td>→</td>
<td>NA</td>
<td>Lateralization of Cx40/Cx43</td>
</tr>
<tr>
<td>Human</td>
<td>Chronic AF &gt;1 y</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
<td>→</td>
<td>Lateralization of Cx40/Cx43</td>
</tr>
<tr>
<td>Human</td>
<td>Chronic AF &gt;5 mo</td>
<td>↓</td>
<td>↑</td>
<td>→</td>
<td>→</td>
<td>Phosphorylated Cx40 ↑</td>
</tr>
<tr>
<td>Human</td>
<td>Chronic AF &gt;6 mo</td>
<td>↓</td>
<td>↑</td>
<td>→</td>
<td>NA</td>
<td>Cx40 ↓ in AF with complex activation</td>
</tr>
<tr>
<td>Human</td>
<td>Lone AF and AF with MVD</td>
<td>↑</td>
<td>NA</td>
<td>↑</td>
<td>NA</td>
<td>Cx40/Cx43 unchanged in lone AF</td>
</tr>
<tr>
<td>Human</td>
<td>Persistent AF &gt;3 mo</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>→</td>
<td>NA</td>
</tr>
<tr>
<td>Human</td>
<td>Chronic AF &gt;3 mo</td>
<td>→</td>
<td>→</td>
<td>→</td>
<td>→</td>
<td>NA</td>
</tr>
<tr>
<td>Human</td>
<td>Chronic AF up to 6 mo</td>
<td>↓</td>
<td>NA</td>
<td>↑</td>
<td>NA</td>
<td>Lateralization of Cx40/Cx43</td>
</tr>
<tr>
<td>Human</td>
<td>Chronic AF &gt;1 y (valve disease)</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>NA</td>
<td>Cx40/Cx43 lateralization.</td>
</tr>
<tr>
<td>Human</td>
<td>Post-operative AF (CAD)</td>
<td>→</td>
<td>NA</td>
<td>→</td>
<td>NA</td>
<td>Cx40/43 reduced in arrested-heart surgery; not beating-heart or in AF</td>
</tr>
<tr>
<td>Human</td>
<td>Persistent AF (MVD, CAD)</td>
<td>→</td>
<td>NA</td>
<td>↑</td>
<td>↑</td>
<td>Lateralization of Cx43</td>
</tr>
<tr>
<td>Human</td>
<td>Permanent AF &gt;3 mo</td>
<td>→</td>
<td>NA</td>
<td>↑</td>
<td>→</td>
<td>Lateralization of Cx43</td>
</tr>
</tbody>
</table>

Abbreviations: ATP, atrial tachypacing; CAD, coronary artery disease; dephos, dephosphorylation; exp, expression; hetero, heterogeneity; SR, sinus rhythm; MVD, mitral valve disease; NA, not available.

Disclosures
None.

References


Key Words: Editorial | antiarrhythmia agents | arrhythmia (mechanisms) | atrial fibrillation | gene expression | gene therapy
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Circulation. 2012;125:203-206; originally published online December 8, 2011;
doi: 10.1161/CIRCULATIONAHA.111.075432

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