Potential targets to reverse atrial remodeling induced by atrial fibrillation

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**Summary**

Atrial fibrillation (AF) is one of the most common cardiac arrhythmias and is an important contributor to cardiovascular morbidity and mortality. The self-perpetuating nature of AF is divided into two major pathophysiological mechanisms: electrical and structural remodeling. Cardiomyocyte stress results in early remodeling, such as contractile dysfunction and changes in action potential duration and later on in down-regulation of L-type calcium channels and structural remodeling, most importantly myolysis, which contribute to contractile dysfunction. The reversal of electrical remodeling is a very fast process, during 3-4 days, in contrast to the reversal of structural remodeling which takes at least several months. Since AF-recurrences also occur one week after cardioversion, the persisting high susceptibility must be a result of the process of structural remodeling. To manage AF, therapies should therefore focus on the reversibility of structural remodeling. Treatment possibilities nowadays focus on rhythm and rate control, which control only electrical properties. To manage AF by improving the arrhythmogenic substrate, future research should focus on drugs that reverse structural remodeling. These potential drugs are angiotensin II type-1 receptor blockers, statins, multi-channel blockers and miRNAs. The action mechanism of these drugs have to be investigated because of their reversible actions on structural remodeling in myocytes.
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Introduction

Atrial fibrillation (AF) is one of the most common cardiac arrhythmias and is an important contributor to cardiovascular morbidity and mortality. AF is a progressive tachyarrhythmia in which the atria lose the control of the sinus node pacemaker, resulting in rapid and irregular activity of the atria. Instead of contracting with a normal rhythm of 60 pulses per minute, the muscle wall only quivers with 400-600 beats per minute.\(^1\)

AF is defined with an self-perpetuating nature because paroxysmal AF often alters chronic AF. Paroxysmal AF is being favored by changes in atrial electrical properties occurring within a few days, a process called ‘electrical remodeling’. The promotion to sustained AF takes at least a few weeks which is the cause of the developing fibrosis, a process called ‘structural remodeling’. Thus, ‘AF begets AF’.\(^1\)

The pharmalogical treatment of AF remains challenging since the currently used antiarrythmia have profound effects on the ventricles. Therefore new drugs that decrease the arrhythmogenic substrate have to be found to challenge the self-perpetuating nature of AF to maintain and initiate itself. Until now, only protective effects of several drugs on the prevention of AF-induced remodeling are studied. Since most patients are diagnosed with AF when they are already suffering from atrial remodeling, it is clinically more relevant to use a drug that reverses the arrhythmogenic substrate with the aim to recover the heart from AF remodeling.\(^2\)

In this report I outline some targets, which may have an reverse effect on AF remodeling and therefore are interesting subjects for new research on AF.
Chapter 1: Atrial remodeling

The self-perpetuating nature of AF is by experimental and clinical studies classified into two major pathophysiological mechanisms: electrical and structural remodeling. These mechanisms are depicted in Figure 1.

Electrical remodeling

In 1995 two independent studies proposed the concept of tachycardia-induced electrical remodeling. In the dog a 15% reduction of the atrial refractory period was found after rapid atrial pacing. In the goat a 45% reduction and a loss or even an inversion of the atrial refractory period was found as a result of a fibrillating pacemaker. Because the shortening of the atrial refractory period was found after long-term pacing, this could be due to changed expression levels of ion channels and therefore was called ‘electrical remodeling’.

Long term rapid atrial pacing is correlated with increased susceptibility to AF, after 2-3 weeks of pacing, 90% of the goat had persistent AF. This led to the concept of ‘Atrial Fibrillation Begets Atrial Fibrillation’ which can be explained by a shortening of the wavelets.

In AF the increased contraction rate of the heart leads to an immediate, followed by a gradual decrease in action potential duration (APD). In patients with chronic atrial flutter or atrial fibrillation, slow pacing of 15 – 30 minutes after electrical conversion lead to an APD of 130 – 150 ms shorter than control patients. Because of this APD reduction, the atrial refractory period is decreased, which leads to a shorter period in which re-entry of an action potential can occur, and thus maintaining AF. This together is called short-term adaptation and goes along with changes in the L-type calcium channel, followed by an calcium overload.

During the first few days of AF, electrical remodeling takes place as a result of atrial cellular stress. With each action potential and inward L-type Ca2+ current (ICaL) enters the cardio myocytes. Because in AF cells fire about a tenfold faster with an irregular rate, a tenfold Ca2+ enters the cells causing the cells to inactivate their L-type Ca2+ channels to protect themselves against an Ca2+ overload. A decreased ICaL results in a decreased length of the action potential and a loss of the contractile function and thus contributes in maintaining AF. Moreover adding BayK, a Ca2+-channel agonist, to rapid paced atrial cells could restore the action potential.

Also a second mechanism contributes to a decreased ICaL. Recently, it was observed that activation of calcineurin, CAMK-II, and protein-phosphatase related pathways adjusted a number of key intracellular proteins involved in ICaL, causing a decrease in ICaL. Besides this, de-phosphorylation of the L-type Ca2+ channels has indirectly proved to be associated with changes in the kinomic profile resulting in ICaL downregulation. Finally, the Ca2+ overload could be maintained because of hyperphosphorylation of phospholamban and ryanodine receptors (RyR2) leading to a Ca2+ leak from the SR.

It is known that cysteine proteases play an important role in initiating and executing apoptosis. Recently, the cysteine protease calpain is found to play an important role between Ca2+ overload and atrial remodeling. Because apoptosis is not always completed in cardiac myocytes, activation of cysteine proteases may be stress intensity dependent. Activated cysteine proteases may therefore be
responsible for L-type Ca\(^{2+}\) channel degradation or cleavage of myofilament proteins (troponin-1, cardiac troponin-T and actin), resulting in a shortened APD, myolysis and contractile dysfunction.  

**Structural remodeling**

In addition to electrical remodeling, AF is also associated with morphologic adaptations and maladaptations. When AF persists over a longer time, ultrastructural alterations of the atrial myocytes are seen which closely resemble the changes of a chronic hibernating ventricular myocardium. The cardiomyocytes turn into a fetal-like phenotype, called dedifferentiation, because of a loss of desmin and the re-expression of α-smooth muscle actine. Dedifferentiation is possibly a result of a physiological adaptation of the tissue to the metabolic stress and the Ca\(^{2+}\) overload.  

These structural changes of the atrial myocytes are heterogeneously distributed with some cells more affected than other and include: (1) an increased cell size, (2) perinuclear glycogen accumulation, (3) loss of sarcomeres (myolysis), (4) changed expression levels of connexin, (5) alterations in mitochondrial shape, (6) loss of sarcoplasmic reticulum, (7) redistribution of nuclear chromatin and (8) altered quantities and localizations of structural cellular proteins. Most protruding is the increased myocyte cell size which is associated with more myolysis and perinuclear glycogen accumulation. Myolysis mostly occurs during chronic AF and this degradation of the myofibril structure enhances the loss of the contractile function.  

Most structural changes are seen in every animal model but there are some differences between different species and different models of atrial fibrillation. In the dog an increased size of the mitochondria is found correlated with a high atrial rate, whereas in the goat numerous much smaller mitochondria were found with longitudinally oriented cristae.  

Also in animal models of pure atrial tachyarrhythmias the extracellular matrix stayed unchanged, whereas in rapid paced and mitral regurgitated dogs the extra cellular matrix increased. Also gap junction remodeling between different species varies. In relation to animal models, more abundant structural changes are found in patients, which might be related to the older age and/or unifying heart diseases.  

Because the morphological changes can’t be defined as ‘degenerative’, apoptosis might not be important during structural remodeling and cardio myocytes are thus kept viable but not functional.  

**Contractile remodeling**

A higher degree of contractile dysfunction is correlated with a longer duration of AF. In a study, the contractile force of the myocytes in chronic AF patients was shown to be reduced by 75%. The mechanism how contractile remodeling occurs is until now not completely understood but this contractile dysfunction seems to be mainly a result of a decreased \(I_{Ca}\).
AF induced atrial remodeling. Cardiomyocyte stress results in a rapid Ca\(^{2+}\) overload, leading to inhibition of L-type Ca\(^{2+}\) channel. Changes in the kinomic profile due to AF leads to indirect inhibition or stimulation of certain proteins. Both early effects lead to a shorter APD and a loss of contractile function. When AF persists, the Ca\(^{2+}\) overload causes calpain activation which degrades target proteins, resulting in myolysis (from Brundel et al.\(^7\)).
Chapter 2: Reverse atrial remodeling

Reverse remodeling in animal models

Ausma et al. investigated the reversibility of structural changes and gap-junctional remodeling in goats with AF, which where cardioversed after 4 months. Previous research has shown that recovery from electrical remodeling occurs within a few days. This study also found recovery of AF cycles lengths (AFCL) and AF atrial effective refractory periods only 2 mo post-AF. In contrast, structural remodeling was not found normalized again 4 mo post-AF. The morphology of the sarcoplasmic reticulum (SR) visible as loose membranes and small mitochondria were comparable to the tissue of goats with AF. Only recovery from the dispersed heterochromatin was found, 4 mo post-AF. The amount of myolysis decreased during post-AF but was still higher than normal goats. The same was found for the ECM but the atrial myocytes diameter is normalized 4 mo post-AF.

A number of structural proteins was studied for their potential to recover. Only cardiotin recovered completely after AF, whereas titin, α-SMA and cell adhesion molecules partially recovered. Also the proteins levels of the gap-juntional Cx40 recovered, where Cx43 remained stable.

Previous research showed that an angiotensin II type 1 receptor blocker (ARB) prevented promotion of chronic AF by suppressing the development of structural remodeling. Therefore Nakashima et al. investigated the reverse remodeling effects of this ARB in dogs with sustained AF and the ability of ARB to suppress AF after sinus rhythm (SR) restoration. They found that ARB had no effect on the atrial effective refractory period but significantly decreased the conduction time (CT). Furthermore, ARB significantly decreased the mean duration of AF compared to the control group and lower percentages fibrosis were found in the ARB treated group compared to the control group.

De Clerq et al. used a horse model for AF to investigate the atrial and contractile remodeling in horses. This group maintained AF by burst-pacing for 7 days and studied a 2-day recovery period. This group once more found a shortening in the atrial effective refractory period and an attenuation of the AERP, as is found in dogs and goats. This electrical remodeling occurred after 4 hours and contractile remodeling after 12 hours of burst pacing. Electrical remodeling was completely reversed after 12 hours, whereas the reversal of contractile remodeling occurred more slowly.

Everett et al. examined in an dog model of chronic AF the contribution of electrical and structural remodeling to the vulnerability to induce AF. In this study, the dogs were rapid atrial paced and with the creation of moderate mitral regurgitation, chronic AF was obtained. They once more found reverse electrical remodeling within 7-14 days post-AF, by looking at the AERP and AFCL. Thereby Lee et al. also found regional differences in the course of reverse electrical remodeling, in that the right atrium faster recovers from shortened and maladapted AERP and from increased inducibility of AF than the left atrium.

On the opposite, several structural changes didn’t recover after sinus restoration: disrupted intercalated disks, sarcomeres at various stages of contraction, glycogen accumulation surrounding the myofibrils, increased number and size of mitochondria and a partially destroyed and indefinite morphology of the SR. Also a loss of some contractile elements was found predominantly around the nucleus.
Reverse remodeling in humans

To compare the reverse electrical remodeling results in animals with humans, Raitt et al.,\textsuperscript{14} investigated electrical changes after cardioversion in human patients with 1-12 months AF. Similar results as in animal studies were found because during the first week of recovery the AERP and the atrial conduction velocity (ACV) increased and the sinus node function improved.\textsuperscript{14}

Structural remodeling of prime importance in AF management

From all this research, see Table 1, electrical remodeling has been shown to be completely reversible within a few days in contrast to the reversal of structural remodeling, which seems to be a very slow process and should take at least several months. Longer post-AF periods have to be investigated to expand our knowledge if even full recovery of structural remodeling could be possible.\textsuperscript{8} During the first week after cardioversion, recurrences of AF still occur and could be related to the process of reverse electrical remodeling. Unfortunately AF-recurrences also occur after 1 week of cardioversion and thus can’t be explained by the process of reverse electrical remodeling. Therefore the continuing high susceptibility to AF might be related to atrial structural remodeling as a result of long-lasting AF. The prime importance in the future to manage AF, is to find new drug therapies that stimulate the reversal of structural remodeling.\textsuperscript{3}

Table 1: Reverse remodeling

<table>
<thead>
<tr>
<th>Species</th>
<th>Duration of AF</th>
<th>Reversibility</th>
<th>Treatment</th>
<th>Study</th>
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<td></td>
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<tr>
<td>Goat</td>
<td>4 wks</td>
<td>Yes</td>
<td>None</td>
<td>Ausma et al,\textsuperscript{9}</td>
</tr>
<tr>
<td>Dog</td>
<td>4 wks</td>
<td>Partly</td>
<td>ARB</td>
<td>Nakashima et al,\textsuperscript{11}</td>
</tr>
<tr>
<td>Horse</td>
<td>1 wk</td>
<td>Yes</td>
<td>None</td>
<td>De Clerq et al,\textsuperscript{12}</td>
</tr>
<tr>
<td>Dog</td>
<td>&gt;8 wks</td>
<td>Yes</td>
<td>None</td>
<td>Everett et al,\textsuperscript{13}</td>
</tr>
<tr>
<td>Human</td>
<td>1-12 mo</td>
<td>Yes</td>
<td>some β-blocker</td>
<td>Raitt et al,\textsuperscript{14}</td>
</tr>
<tr>
<td>Structural</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goat</td>
<td>4 wks</td>
<td>Partly</td>
<td>None</td>
<td>Ausma et al,\textsuperscript{9}</td>
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<tr>
<td>Dog</td>
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<td>&gt;8 wks</td>
<td>No</td>
<td>None</td>
<td>Everett et al,\textsuperscript{13}</td>
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<tr>
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<td>1 wk</td>
<td>Partly</td>
<td>None</td>
<td>De Clerq et al,\textsuperscript{12}</td>
</tr>
</tbody>
</table>
Chapter 3: Potential treatment targets

Atrial fibrillation has over the past years been treated with pharmacological drugs, antiarrhythmia, and non-pharmacological therapies, surgical intervention, percutaneous intervention, targeting ganglionated plexi, ‘ablation therapy’ and implantable devices which control electrical discharges. Unfortunately these treatment possibilities are far from optimal because our understanding of the mechanism of AF is still inadequate. Mainly pharmacologic therapy is an essential component in the treatment of AF because not all patients are capable of undergoing invasive procedures, which are also at this time not globally efficacious. Most antiarrhythmic drugs are designed for controlling ventricular rate and have therefore profound effects on the ventricular electrophysiology. Recently, Class IA, IC and III are found to have beneficial effects of sinus rhythm control but also have increased adverse effects and even an increased mortality rate for class IA drugs.

The electrical changes induced by AF are discussed to rapidly recover after conversion to sinus rhythm. In contrast, structural changes persist, improving the arrhythmogenic substrate, and make the patient vulnerable to AF induction. Therefore new potential drugs to reverse atrial remodeling, with emphasis on reverse structural remodeling, are discussed and listed in Table 2.

Angiotensin II type 1 receptor blockers

Several studies have until now documented that the renine-angiotensin system (RAS) is overexpressed in AF. High levels of the angiotensin-converting enzyme (ACE) and the angiotensin-II (AT-II) receptors are found in patients with AF. Thereby the angiotensin-converting enzyme inhibitor (ACEI) is correlated with a lower incidence of AF in patients with ventricular dysfunction after infarction. Therefore RAS-blockade may be a new therapeutic target for treatment of AF. ACEi and ATII type 1 receptor blockers (ARB) have been documented to be such possible targets.

Nakashima et al treated dogs with olmesartan during 4 weeks of recovery of sustained AF. During recovery, AERP fully recovered in the olmesartan and the control group and CT only recovered in the olmesartan group. Structural changes were investigated by inspecting histological sections of the left atrial free wall. Tissue from the control group showed distributed amounts of interstitial fibrosis and heterogeneous distributed mitochondria varying in size. Quantitative analysis proved that olmesartan treatment significantly attenuated the amount of fibrosis, Figure 2. Olmesartan therefore has reverse structural remodeling properties.

Liu et al examined the preventive effect of irbesartan on chronic ionic remodeling. They found that irbesartan did prevent against shortening of the APD and reduction of APD rate adaptation, although no reduced I\textsubscript{Ca} were found. Although preventive effects against electrophysiological changes have been found, irbesartan has no effect on L-type calcium channels. No reverse remodeling effects of irbesartan have been documented until now.
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Figure 2 Percentage of fibrosis in the left and right atria of the appendages and free walls after 4 weeks of recovery. In all the regions of the atria, the percentage of fibrosis was lower in the olmesartan group than the control group. White bars: control group, black bars: olmesartan-treated group. RAA: right atrial appendage, RAFW: right atrial free wall, LAA: left atrial appendage, LAFW: left atrial free wall. *p<0.001; §p<0.01 compared to the control group (from Nakashima et al.10).

Figure 3 Effects of control SHR, L-NAME, L-NAME + low dose valsartan (L-VAL) and L-NAME + high dose valsartan (H-VAL) treated SHR in left ventricular tissue. A) Treatment with valsartan reduced the percentage L-NAME induced cardiac fibrosis in a dose-dependent manner. B) The mRNA expression of caspase-3 was increased in the L-NAME group compared to the control group and further increased in the L-VAL and H-VAL group. C) Treatment with H-VAL reduced the L-NAME induced mRNA expression of TGF-β1 to the level comparable to the control group. **p<0.01 vs control; ††p<0.01 vs L-NAME; *p<0.05; †p<0.05 (from Akashiba et al.18).

Akashiba et al,18 studied the effect of a chronic angiotensin II type 1 blockade using valsartan on tissue remodeling of the left ventricle in SHR (spontaneously hypertensive rats). The effect of valsartan on mRNA expression levels of TGF-β1 and caspase 3 were also taken in account. Previously, TGF-β1 has been shown to be produced in cardiac cells, vascular smooth muscle cells and fibroblasts promoting fibrosis in cardiovascular tissue and caspase 3 has been shown to be pivotal in caspase protease family, which mediate cellular apoptosis. This study group previously observed hypertensive rats given N⁶-nitro-l-arginine methyl ester (L-NAME), which is inhibits nitric oxide synthetase (NOS), resulting in injuries to hypertensive organs. Therefore to induce fibrosis, L-NAME is given with the studied ARB, valsartan, in a low-dose (L-VAL, 1mg/kg/day) and a high-dose (H-VAL, 30mg/kg/day). The results of this study are shown in Figure 3. Treatment with L-VAL decreased the percentage of fibrosis induced by L-NAME and elevated mRNA caspase-3 levels but did not significantly reduce the mRNA expression levels of TGF-β1. H-VAL in contrast lowered both the expression levels TGF-β1 and caspase-3 and also lowered the blood pressure, which are all thought to contribute to the inhibition of cardiac fibrosis.18
Brundel et al.,\textsuperscript{7} showed that in AF calpains play an important role in atrial myolysis, cell death and contractile dysfunction. Subsequently Sandmann et al.,\textsuperscript{19} found that upregulated levels of cardiac calpain I and II could be reduced by ramipril and valsartan, respectively an ACEi and an ARB.

Therefore Li et al.,\textsuperscript{20} investigated the effect of cilaprazil (ACEi) or valsartan on atrial calpain expression levels and atrial structural remodeling in chronic rapid atrial paced dogs. Mongrels dogs were treated with cilaprazil or valsartan 1 week before and 6 weeks during rapid atrial pacing. Both compounds inhibited calpain-I upregulation but more interestingly, reduced the area of myolysis. Rapid atrial tachypaced dogs showed substantial numbers of atrial myocytes affected by myolysis, which could be reduced by treatment with cilaprazil or valsartan, although the number of affected myocytes still remained higher than in the control group. The myolytic area was in the rapid atrial tachypaced group significantly higher than in the sham group (24.3\% vs. 3.1\%, \textit{p}<0.01), but was significantly decreased in the cilaprazil and valsartan group (\textit{p}<0.05). The results of electron microscopic analysis of the ultrastructure of the myocytes are shown in Figure 4. In normal atrial tissue, a highly organized sarcomeric structure throughout the cytoplasm is apparent consisting of normal-sized mitochondria placed in rows and nuclei with clustered chromatin. Rapid atrial tachypacing severely altered this organized ultrastructure into disintegration of myofilaments, mitochondrial swelling with a decreased density and organization of the cristae and pyknotic nuclei with chromatin margination to the nuclear membrane indicating cell apoptosis. Treatment with cilaprazil and valsartan radically suppressed the ultrastructural changes induced by 6 weeks of rapid atrial tachypacing.\textsuperscript{20}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Transmission electron micrographs of atrial tissue. Normal atrial myocytes show a regular sarcomeric organization with uniformly sized mitochondria and normal clumping of chromatin at the nuclear membrane. A) Tissue from the control group showing a severely altered ultrastructure including disintegration of myofilaments (B), mitochondrial swelling with a decreased density and organization of the cristae (C), pyknotic nuclei with chromatin margination to nuclear membrane indicating cell apoptosis (D). Treatment with cilaprazil (E) or valsartan (F) radically suppressed the chronic tachypaced induced ultrastructural changes. Magnification A, B, D, E, F: 6000x and C: 10000x. (from Li et al.\textsuperscript{20}).}
\end{figure}
Kumagai et al.\(^\text{21}\) studied the effect of candesartan on chronic structural remodeling after the previous finding that candesartan prevents against acute electrical remodeling. Sustained AF was induced in twenty dogs and 1 week before and during pacing, candesartan was orally administered. Besides the observed reversibility of electrical remodeling, a lower percentage of interstitial fibrosis was observed in the candesartan group compared to the control group by analyzing histological sections of each group. The myocytes of the sham group showed a normal distribution of sarcomeres throughout the cell and a normal intracellular space. The myocytes of the control dogs showed a loss of some contractile materials, abnormal sarcomeres, extensive interstitial fibrosis and thick fibrous layers were found in the endo- and epicardium. Also an increase in connective tissue was observed, which was situated around parenchymal cells. These pathological abnormalities were attenuated in the candesartan treated dogs (see Figure 5). Candesartan can therefore prevent AF recurrences by suppressing the development of structural remodeling.\(^\text{21}\)

**Figure 5** Histological sections of the right atrial free wall from a sham dog (A), a control dog (B) and a candesartan treated dog (C). The sham dog showed a normal intracellular space. The control dogs showed extensive interstitial fibrosis, which was attenuated by candesartan. Magnification: 400x, Masson trichrome staining (from Kumagai et al.\(^\text{21}\)).

Finally, the RAS seems to play an important role in AF and seems to have several potential treatment targets to reverse electrical and structural remodeling. An important signaling pathway involved, was found by Tsai et al.\(^\text{22}\) Previously, the Janus kinase (JAK)/signal transducers and activators of transcription (STAT) pathway are shown to mediate the effect of angiotensin II on the structure of a tissue. However the status of Ang II/JAK/STAT signaling and its role in atrial structural remodeling is unknown. Therefore Tsai et al.\(^\text{22}\) cultured atrial myocytes and found that angiotensin II activates STAT3 via Rac1 and induces atrial fibrosis. To study the effect of angiotensin II on the structure of the atrial tissue in vivo, Wistar rats were infused with angiotensin II for 6 hours, 3 days or 14 days and cotreated with orally given losartan or simvastatin (a statin) each day during angiotensin II infusion. Light microscopic pictures of left atrial tissue, which were 14 days infused, were analyzed, see Figure 6. The angiotensin II infused group showed more extracellular matrix accumulation and fibrosis within the intercellular space and pericardial thickening, than the control group. Cotreatment with losartan or simvastatin attenuated these structural changes and therefore losartan and simvastatin may be able to reverse structural remodeling.\(^\text{22}\)
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**Figure 6** Light microscopic pictures of atrial tissue of the control, Ang II infused, Ang infused+losartan and Ang II infused+simvastatin group, stained with Masson’s trichome staining. Compared to the control group (A and B), the Ang II infusion group shows more extracellular matrix accumulation and fibrosis within the intercellular space (C) and in the pericardium (D). Co-treatment with losartan (E and F) or simvastatin (G and H) attenuated this amount of damage. 200x magnification and n=3 (from Tsai et al.⁵¹).

**Statins**

The presence of and the risk at AF has been correlated with a systemic inflammation and also tissue from AF suffers from oxidative stress.⁵² Hydroxymethylglutary coenzyme A reductase inhibitors (statins) have been shown to have pleiotropic effects, such as anti-inflammatory and antioxidant properties, and to have preventive effects against atrial fibrillation induced remodeling.⁵³ For example atorvastatin has preventive electrical and structural remodeling actions.⁵⁴ Simvastatin prevents AF promotion by reducing Ca,1.2 protein expression and reducing the decrease in AERP.⁵⁵ Chao et al,⁵⁶ pre-treated rabbits with simvastatin to examine the anti-arrhythmic effect of simvastatin on membrane ionic currents in left ventricular myocytes and found attenuated electrical changes compared to the control group. Therefore statins could be new drugs to reverse atrial remodeling.⁵⁶

**Multi-channel blockers**

Bepridil is a multi-channel blocker, which has been shown to restore and maintain sinus rhythm in patients with long-lasting persistent AF.⁵⁶ Therefore, Nishida et al,⁵⁷ investigated the effect of bepridil on reverse electrical remodeling in dogs after rapid atrial tachypacing. They found that bepridil could reverse at least some electricalphysiological remodeling effects such as, prolonging AERP and tachycardia-induced L-type calcium channel down regulation.⁵⁷

Amiodarone is a multi-channel blocker that blocks multiple ion-channels and alpha- and beta- adrenergic receptors and also has anti-inflammatory actions. Amiodarone is a valuable drug in terminating and preventing the recurrence of persistent AF but the effect of amiodarone on electrical and structural remodeling is unknown. Therefore Ashikaga et al,⁵⁸ rapid atrial and ventricular paced dogs for 6 weeks followed by or atrial or ventricular pacing for 4 weeks with or without in combination with amiodarone treatment. Compared to dogs without amiodarone, amiodarone treatment led to APD and AERP restoration and recovery of loss of normal rate
adaptation and of action potential duration. This study also looked at matrix metalloproteinases (MMPs), which are zinc-dependent endopeptidases and serve as a marker for structural remodeling. MMP-2 activity was increased in the atrial paced group compared to the control group and was significantly decreased in the amiodarone treated group. Histological sections confirmed this finding because amiodarone treatment could reduce the percentage of fibrosis induced by atrial pacing. Amiodarone treatment also suppressed fibrosis and therefore reversed both electrical and structural remodeling. 28

Nifekalant (NIF) is an K channel blocker and is an antiarrhythmic agent permitted in Japan to treat arrhythmias and ventricular tachycardia. Because of previous found preventive effects of NIF against AF, 29 Tang et al, 30 treated dogs with NIF after 24 hours of rapid atrial pacing. They found that NIF inhibited ERP shortening and ERP heterogeneity increasing and conclude therefore that NIF has reverse acute electrical remodeling properties.

**Micro-RNAs**

Previously, connective tissue growth factor (CTGF), a protein that stimulates ECM synthesis, is found to be an important regulator of fibrosis in different pathologies in different organs, including the heart. In fibroblasts and cardiac myocytes, transforming growth factor β (TGFβ) and other prohypertrophic stimuli are shown to regulate CTGF expression. microRNAs (miRNAs) could also have fibrosis regulating properties because they are posttranscriptional regulators of gene function and they play an important role in growth, development and stress responses of the heart. Duisters et al, 31 investigated whether miRNAs can regulate genes involved in fibrosis in the heart. From bioinformatical research miR-133 and miR-30 seem to be the best miRNAs that target CTGF, which both are also down-regulated in several heart pathologies. First, in two animal models, a rat model for hypertension-induced left ventricular hypertrophy and a mouse model for transverse aortic constriction-induced hypertrophy accompanied by fibrosis, the levels of miR-133 and mi-30 were inversely correlated to the amount of CTGF mRNA and protein. Second, in cultured cardiomyocytes and fibroblasts, mi-R133 and mi-R30 knockdown resulted in increased CTGF levels by more than 100% and 300% for respectively CTGF mRNA and protein level. Third, Overexpression of both miRNAs resulted in down-regulation of CTGF, accompanied by a decreased production of collagen 1 and fibronectin, see Figure 7. 31

**Figure 7** Relative overexpression of collagen type 1 (col1a1 and col3a1) and fibronectin in cultured cardiac fibroblasts. Knockdown of endogeneous miR-30 results in enhanced expression of collagen type 1 and fibronectin. *p<0.05 compared to control-treated cells (from Duisters et al. 31)

For the miR-29 family is known that it is down-regulated in regions of the heart next to an infarction and that this family targets mRNA of proteins involved in fibrosis including several collagens, fibrillins and elastin. Therefore van Rooij et al, 32 investigated if down-regulation of the miR-29 family results in increased levels of these mRNAs and stimulates the fibrotic process. The expression of predicted
miR-29 mRNA targets was measured using RT-PCR in cardiac samples 3 days after an acute myocardial infarction (MI) due to coronary artery occlusion. Down-regulation of miR-29 using anti-miR correlated with up-regulation of collagen 1 and 3 and fibrillin in the infarcted region and correlated with elastin in the remote myocardium (Figure 8).

This broaches a new subject that miRNAs could play an important role in structural remodeling in the myocardium. Possibly, small silencing RNAs or antisense nucleotides could be new subjects in future research with the aim to develop novel drugs for patients with AF.

**Figure 8** Regulation of ECM proteins by miR-29. Real time PCR analysis of predicted genes in the region of the infarcted (borderzone) and the non-infarcted region (remote) of the myocardium 3 days after MI. Downregulation of miR-29 correlates with an increase in collagens (COL1A1, COL1A2, COL3A1) and fibrillin (FBN1) but did not correlate with elastin (ELN1). N=3-4 *p<0.05 compared with sham operated animals, †p<0.05 compared with luciferase reporter alone (from van Rooij et al.)
### Table 2: Treatment possibilities to reverse atrial remodeling

<table>
<thead>
<tr>
<th>Drug</th>
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Discussion

Main Findings

AF is a common arrhythmia with a self-perpetuating nature supported by the process of electrical and structural remodeling. Cardiomyocyte stress results in early remodeling, such as contractile dysfunction and changes in action potential duration and later on in down-regulation of L-type calcium channels and structural remodeling, most importantly myolysis, which contribute to contractile dysfunction. Most patients are diagnosed with AF when they are already suffering from atrial remodeling. Unfortunately, treatment nowadays consist of rhythm and rate control and only preventive actions of several drugs against atrial remodeling are seen. Therefore it is clinically very relevant to find new drugs that reverse atrial remodeling.

Studies investigating the reversibility of atrial remodeling showed that the reversal of electrical remodeling is a fast process. Recovery from AF cycle lengths, atrial effective refractory period and atrial conduction velocity occurred within a few days until a few weeks. The reversal of structural remodeling on the other hand seems a very slow process and takes at least several months. Structural changes due to atrial fibrillation include disrupted intercalated disks, sarcomeres at various stages of contraction, glycogen accumulation surrounding the myofibrils, increased number and size of mitochondria and a partially destroyed and indefinite morphology of the SR.

After cardioversion, the high susceptibility to AF remains high because recurrences still occur more than one week after cardioversion. Since electrical remodeling is easily reversed within a few days, recurrences must be related to structural remodeling. Therefore to manage AF, the prime importance of future research is to find drugs that can reverse structural remodeling. In this report several drugs have been described to be potential drugs in reversing structural remodeling including angiotensin II type-1 receptor blockers, statins, multi-channel blockers and miRNAs. The angiotensin II type 1 receptor blockers olmesartan, valsartan, candesartan and losartan are shown to reverse structural remodeling. Olmesartan lowered the percentages of fibrosis in the left and right atria of the appendages and free walls after 4 weeks of recovery. Treatment with valsartan resulted in decreased percentages of fibrosis and also decreased the expression level of TGF-β and caspase-3. Candesartan attenuated the structural changes found in the tachypaced dogs including a loss of contractile materials, abnormal sarcomeres, extensive interstitial fibrosis and thick fibrous layers in the endo- en epicardium. Finally losartan and also the statin simvastatin attenuated the structural changes found in the angiotensin II infused group including extracellular matrix accumulation and fibrosis within the intercellular space and pericardial thickening. The multi-channel blocker amiodarone decreased the level of MMP-2 activity, induced by atrial pacing, and reduced the percentage of fibrosis. miRNAs could also have regulating properties because they are posttranscriptional regulators of gene function. Up-regulation of miR-133 and miR-30 seem to down-regulate the profibrotic gene CTGF. Up-regulation of miR-29 resulted in down-regulation of collagen 1 and 3 and fribillin.

It appears clear that the action mechanism of these drugs remain unknown but have to be investigated because of their reversible actions on structural remodeling, to develop new approaches in the treatment of AF.
**Angiotensin-related electrical alterations**

ATII has been shown to enhance electrical remodeling, which was completely reversed by angiotensin II type 1 receptor blockade. Previously, also treatment with an ACE-inhibitor showed reverse electrical remodeling effects, which suggests that ATII exerts its effect trough ACE-dependent pathways (and not for example by chymase-dependent ATII configuration).

The most important effect of ATII blockade on electrical remodeling seems to be AERP shortening in paroxysmal AF, however in chronic AF, AERP shortening seems to independent of ATII. In animal models of chronic AF, ATII is correlated to changes in intra-atrial conduction time. Therefore ATII exerts an effect during the prolongation of paroxysmal to persistent AF.

The underlying ionic mechanism by which ATII has an effect on the atrial vulnerability is still unknown. ATII could have an effect on ionic exchange resulting in an calcium overload, trough a mechanism by which the calcium release from the endoplasmatic reticulum through activation of the inositol-1,4,5-triphosphate pathway is increased as well as by which the extracellular calcium intake is increased. Also protein kinase C, which is activated by ATII, phosphorylates L-type Ca\(^{2+}\) channels resulting in an increased Ca\(^{2+}\) influx, which is conflicting with other generally observed results of a decreased L-type Ca\(^{2+}\) influx. The Ca\(^{2+}\) channel activity appears to be a balance of regulating kinases (increased I\(_{Ca}\) trough phosphorylated Ca\(^{2+}\) channels) and phosphatases (decreased I\(_{Ca}\) trough dephosphorylated Ca\(^{2+}\) channels). In chronic AF this balance is thus shifted towards the phosphatases. In general, ATII is part of a system of activators, inhibitors and mediators involved in Ca\(^{2+}\) exchange, which affects other ion concentrations as well.\(^{16}\)

**Angiotensin-related structural alterations**

The renine-angiotensin system has been shown to be related to interstitial fibrosis in AF and some of the underlying mitogenic signaling pathways have been investigated including MAPKs (mitogen-activated protein kinases) and JAK/STAT (janus kinases / signal transducer and activator of transcription). Expression levels of members of MAPK subfamily, extracellular signal-regulated kinases1 and 2 (ERK 1 and 2), c-Jun-N-terminal kinase (JNK) and p38-MAPKs, have been studied. ERK 1&2 activate transcription factors, which are involved in the expression of structural and cell-cycle regulatory proteins, eventually leading to cellular growth and proliferation. However ERK, JNK and p38 all were all upregulated, only ERK levels were correlated to ATII levels. Also did ACE-inhibition not prevent upregulation of JNK and p38, implying JNK and p38 to have a stress-responsive role and ERK to be the main pathway activated by ATII. Further investigation of the ERK pathway revealed the proapoptotic protein Bax, the antiapoptotic protein Bcl and the apoptosis-related protease caspase-2 activity, involved in this pathway. Confirming the hypothesis that RAS activity leads to apoptotic changes, ACE inhibition decreased proapoptotic proteins levels and prevented apoptosis. However, ACE inhibition did to change the overall cell-death rate which proves that the development of fibrosis is mediated by ATII-dependent (Figure 9) and ATII-independent pathways.\(^{16}\)
Figure 9 Atrial structural alterations caused by an ATII-dependent pathway. ATII: angiotensin II, MEK1/2: ERK activating kinases 1 and 2, ERK1/2: extracellular signal-regulated kinases (from Dilaveris et al.16)

MiRNAs

Around the region of a myocardial infarction miR-29 is downregulated but seems to have a key role in the control of cardiac fibrosis. The precise mechanism behind this down-regulation is not completely determined but it seems that TGFβ, an important regulator of cardiac fibrosis, can repress miR-29 expression. Also BNP from cardiomyocytes, which is secreted in stressful conditions, has been shown to influence TGFβ signaling and therefore also modulates miR-29 expression. A model for the role of miR-29 in cardiac fibrosis is shown in Figure 10. TGFβ has been of great interest as a new therapeutic target because of its role in fibrosis. Unfortunately, the problem was that TGFβ has also some other functions for example in the immune response. To control fibrosis, miR-29 seems a novel target but it is important to remind that miR-29 downregulation did not upregulate elastin expression. Also a few-fold decrease in miR-29 resulted in a 20-fold increase in collagens and fibrillin, whereas a miR-29 antagomir induced collagens only to some extent. This means that the presence of a mRNA binding site not only determines the targeting of mRNA by miR-29 but also other regulatory steps of miR-29 are involved in the control of cardiac fibrosis.32

Figure 10 A model for the role of miR-29 in cardiac fibrosis. As a result of cardiac stress, TGFβ is activated and stimulates down-regulation of miR-29. Through the loss of inhibition, collagens and other ECM proteins are expressed, leading to fibrosis. Also as a result of cardiac stress, anti-BNP is secreted from the cardiomyocytes, which influence the activation of BNP and therefore influence the profibrotic actions of TGFβ (from van Rooij et al.32)
Limitations

Experimental animal models are important tools for investigating AF because they largely mimic the in vivo situation in patients with AF. Unfortunately, patients often suffer from underlying heart diseases, a situation incomparable to most models which mostly represent ‘lone’ AF. Possible influences from these underlying heart diseases on the clinical picture of AF remains therefore unknown. Recent research made clear an important difference between RAS blockade in patients with lone AF and patients with structural heart diseases. In a burst-paced goat model of lone AF, candesartan was not able to reverse electrical remodeling. This study supports the hypothesis that RAS blockade mainly acquires its positive effects of improvements in underlying heart diseases rather than antiremodelling effects.

Also suffer patients for many years of AF and occurs AF much more at elderly age, a situation which is hard to mimic in animal models. Despite these limitations, experimental models greatly increase our knowledge about AF and improvement of these models are of great importance in understanding AF.
References