Chapter 2

Loss of proteostatic control as a substrate for Atrial Fibrillation; a novel target for upstream therapy by Heat Shock Proteins

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Abstract

Atrial Fibrillation (AF) is the most common, sustained clinical tachyarrhythmia associated with significant morbidity and mortality. AF is a persistent condition with progressive structural remodeling of the atrial cardiomyocytes due to the AF itself, resulting in cellular changes commonly observed in ageing and in other heart diseases.

While rhythm control by electrocardioversion or drug treatment is the treatment of choice in symptomatic AF patients, its efficacy is still limited. Current research is directed at preventing first-onset AF by limiting the development of substrates underlying AF progression and resembles mechanism-based therapy. Upstream therapy refers to the use of non-ion channel anti-arrhythmic drugs that modify the atrial substrate- or target-specific mechanisms of AF, with the ultimate aim to prevent the occurrence (primary prevention) or recurrence of the arrhythmia following (spontaneous) conversion (secondary prevention).

Heat shock proteins (HSPs) are molecular chaperones and comprise a large family of proteins involved in the protection against various forms of cellular stress. Their classical function is the conservation of proteostasis via prevention of toxic protein aggregation by binding to (partially) unfolded proteins. Our recent data reveal that HSPs prevent electrical, contractile and structural remodeling of cardiomyocytes, thus attenuating the AF substrate in cellular, and animal experimental models. Furthermore, studies in humans suggest a protective role for HSPs against the progression from paroxysmal AF to persistent AF and in recurrence of AF.

In this review, we discuss upregulation of the heat shock response system as a novel target for upstream therapy to prevent derailment of proteostasis and consequently progression and recurrence of AF.
1. Management of atrial fibrillation by upstream therapy

Atrial fibrillation (AF) is the most common clinical tachyarrhythmia which significantly contributes to cardiovascular morbidity and mortality, mainly through stroke and heart failure. The incidence of AF is escalating due to the increased prevalence of risk factors constituting a substrate for AF, such as obesity\(^1\), metabolic syndrome\(^2\) and increasing age.\(^3\) In addition to the increased first-onset of AF, also the progression of the arrhythmia poses problems, as the longer AF persists the less effective pharmacological and electrical cardioversion therapies are.\(^4\) In patients with symptomatic AF, rhythm control is the treatment of choice.\(^5\) However, the effective reversal to sinus rhythm is still inadequate. Therefore, recent research focuses on the identification of mechanisms underlying AF substrate induction and maintenance, which have led to several novel upstream therapeutic approaches, including angiotensin-converting enzyme inhibitors, aldosterone antagonists, statins and polyunsaturated fatty acids.\(^6\) Upstream therapy refers to the use of non-ion channel anti-arrhythmic drugs that modify the atrial substrate- or target-specific mechanisms of AF with the ultimate aim to prevent the occurrence (primary prevention) or recurrence (secondary prevention) of the arrhythmia.\(^7,8\)

It has been recognized that electrical and structural remodeling of cardiomyocytes create a substrate for AF.\(^9\) Nevertheless, the exact molecular mechanisms that underlie cardiomyocyte remodeling and AF progression are as yet unidentified. We recently obtained evidence that derailment of proteostasis (i.e. the homeostasis of protein production, breakdown and function) constitutes an important substrate for induction and progression of AF.\(^10-12\) Proteostasis involves controlling the concentration, conformation, binding interaction, kinetics and location of individual proteins. Derailment of cellular proteostasis results in many systemic diseases including cardiovascular disorders.\(^13\) Cells respond to a loss of proteostatic control by inducing the heat shock response (HSR) resulting in the expression of heat shock proteins (HSPs) that facilitate protein folding and function.\(^14\) Consequently, an emerging target candidate for upstream therapy of AF is the upregulation of HSPs. Indeed, HSP induction alleviates the occurrence and recurrence of AF in various experimental model systems for AF.\(^12,15-17\) Furthermore, studies in humans suggest a protective role for HSPs against progression from paroxysmal AF to persistent AF\(^15\) and the restoration of sinus rhythm in patients with persistent AF (secondary prevention).\(^18\)

Here we discuss the concept that derailment of cardiomyocyte proteostasis constitutes an important aspect of the substrate for AF. In addition we examine the evidence for induction of the HSR system as a novel target for upstream therapy to prevent the occurrence and the recurrence of AF and address its possible modes of action.
2. Mechanisms underlying AF initiation and progression; derailment of proteostasis

Although the exact molecular mechanism(s) underlying AF initiation, maintenance and progression has not yet been completely elucidated, an important recognition was that AF induction required a suitable substrate as well as a trigger that acts on the substrate. Various clinical conditions, e.g. several heart diseases, hypertension and cardiac surgery, are risk factors for the first-onset of AF, as they create a substrate(s) and/or trigger(s) for the initiation of AF (Figure 1). Key AF promoting factors have been identified, including inflammation, oxidative stress, active Rho-GTPase, fibrosis and atrial muscle bundle dissociation, which induce loss of proteostatic control, creating a substrate for AF. Subsequent triggers will act on the substrate and will induce AF.

![Figure 1: Overview of AF-promoting factors contributing to first-onset AF.](image)

Once AF is initiated, AF itself will induce further electrical and structural remodeling in a manner that contributes to AF maintenance and progression. A conceptual model is depicted in Figure 2. Electrical remodeling resulting in shortening of action potential duration (APD), slowing of conduction and abnormal calcium handling will favor AF maintenance. When AF persists, the calcium overload causes irreversible changes in structural remodeling, especially cardiomyocyte hibernation. Hibernation is characterized by irreversible degradation of the myofibril structure (myolysis) and mitochondrial damage, implying impaired energy production and release of reactive oxygen species, which leads to contractile dysfunction. Other characteristics are redistribution of nuclear chromatin and pathological gene expression revealing a deficiency in healthy
cardiomyocyte proteostasis. While early electrical remodeling is reversible, the derailment of proteostasis underlies irreversible structural remodeling and thereby AF progression. We and others identified various molecular mechanisms that underlie derailment of proteostasis and AF progression and recurrence.

**Figure 2**: Overview of AF-induced derailment of cardiomyocyte proteostasis. AF induces time-related progressive myocyte remodeling. First, AF causes cellular Ca$^{2+}$ overload and oxidative stress, which results in a direct inhibition of the L-type Ca$^{2+}$ channel, shortening of action potential duration and contractile dysfunction. These changes have an early onset and are reversible. The early processes protect the cardiomyocyte against Ca$^{2+}$ overload but at the expense of creating a substrate for persistent AF. When AF persists, derailment of proteostasis occurs via activation of calpains, kinases/phosphatases, RhoA-GTPase, and exhaustion of protective HSPs. The key modulators also activate each other. Derailment of proteostasis results in irreversible myolysis/hibernation, alterations in structural proteins and pathological gene expression, which are substrates for impaired contractile function and AF persistence. Upstream therapies are directed at modifying the substrate for AF progression. Normalization of the cardiomyocyte proteostasis by inducing HSP expression might represent an effective approach to manage clinical AF.

### 2.1. Ion channel currents and calcium handling

During AF, atrial cardiomyocytes are subjected to very rapid (400-600 times per min) and irregular firing, causing a rapid Ca$^{2+}$ overload resulting in functional down-regulation of the L-type Ca$^{2+}$ channel, which leads to shortening of the action potential duration and contractile dysfunction (hypocontractility), thus providing a further substrate for AF (Figure 2). Decreased $I_{CaL}$ has been consistently found in atrial remodeling, which is believed to significantly contribute to electrical remodeling and AF progression.
functional changes have a rapid onset following initiation of AF and are reversible. In addition to the L-type Ca\(^{2+}\) channel, also other channel currents are affected, as previously reviewed. Ion channel currents are regulated by expression level, phosphorylation and redox status of the channel, all of which are altered during AF.

In dog atrial cardiomyocytes, tachypacing induced the activation of calcineurin via the Ca\(^{2+}\)/calmodulin system, which in turn changes cardiomyocyte proteostasis by stimulating nuclear translocation of NFAT, resulting in transcriptional and translational downregulation of the L-type calcium channel resulting in atrial remodeling and AF progression. Phosphorylation status of ion channels are altered due to changes in activity of various kinases and phosphatases, such as enhanced CAMKII, PP1 and PP2a activity. Further, oxidative stress during AF can contribute to changes in redox status, thereby altering ion channel currents.

2.2. Kinome activity and target proteins

Kinome is a global description of kinases and kinase signaling. Various kinases and phosphatases become activated during AF and contribute to AF-induced remodeling and contractile dysfunction. In addition to modification of ion channel currents through (de)phosphorylation, also the function of other downstream target proteins are affected by the altered activity of kinases and phosphatases, including calcium handling proteins, such as RyR2 and PLB. Modification of calcium handling protein function will contribute to calcium overload and subsequent contractile dysfunction. Furthermore, also kinases involved in post-translational modifications of structural proteins, such as actin, have been implicated in AF-induced remodeling. Tachypacing-induced activation of RhoA-GTPase and downstream Rho-kinase (ROCK), induces F-actin stress fiber formation (Figure 2). Prevention of stress fiber formation by ROCK-inhibition or HSP expression prevented tachypacing-induced remodeling. These findings imply a key role for alterations in kinome in causing derailment of proteostasis and progression of AF.

2.3. Protease activity

The cysteine protease calpain is activated during AF. Calpain is persistently activated by the AF-induced intracellular Ca\(^{2+}\) overload, which results in degradation of contractile and structural proteins, leading to myolysis, thereby further contributing to irreversible structural remodeling and AF progression.

Thus, AF-induced derailment of proteostasis includes changes in ion-channel function, kinome, and calpain activation and underlies reversible electrical remodeling and irreversible structural remodeling and thereby AF initiation and progression.
3. Heat shock proteins protect against AF initiation and progression

It has been recognized that heat shock transcription factor 1 (HSF1) is an important regulator of proteostasis by controlling the expression of major HSPs, including HSPB1 (HSP27), HSPA1A (HSP70) and HSPC1 (HSP90), that facilitate protein folding, localization and function.\textsuperscript{70,71} Induction of HSPs provides cytoprotective effects against stress induced derailment of proteostasis and is beneficial in various cardiac diseases (Table 1).\textsuperscript{46,48,70-83} Therefore, recent studies have investigated the cardioprotective potential of HSPs in AF, focusing on AF induction as well as progression.

Table 1: Major cardioprotective heat shock proteins, localization, expression and cardiac disease protective effects.

| Family name | Protective member (alternative name) | Cardiac disease | localization | Cardiac Expression | References |
|-------------|--------------------------------------|-----------------|--------------|--------------------|------------|
| HSPA | HSPA1A (HSP70) | ischemic heart disease, hypertrophy | cytosol | +++ | 72,73,78 |
| DNAJ | DNAJA3 (HSC40) | dilated cardiomyopathy | cytosol/nuclear | +++ | 76 |
| DNAJB5 (HSP40) | hypertrophy | cytosol/nuclear | +++ | 82 |
| HSPB | HSPB1 (HSP25, HSP27,HSP28) | AF, ischemic heart disease | cytosol | +++ | 46,48,74,79 |
| | HSPB5 (αβCrystallin, CRYAB,CRYA1) | (dilated) cardiomyopathy | cytosol | ++++ | 77,81 |
| | HSPB6 (HSP20, p20) | AF, ischemic heart disease | cytosol | ++ | 48,83 |
| | HSPB7 (cvHSP) | AF | cytosol | ++++ | 48 |
| | HSPB8 (HSP22,H11) | AF | cytosol | ++ | 48 |
| HSPD | HSPD1 (HSP60) | heart failure | mitochondria | ++++ | 80 |
| HSPC | HSPCA (HSP90) | ischemic heart disease | cytosol | ++++ | 75 |

4. HSPs in the prevention of first-onset AF (primary prevention)

HSPs protect against a variety of AF-promoting factors contributing to first-onset AF (Figure 1). Protective effects of HSPs against cell death, fibrosis, Rho-GTPase activation, oxidative stress and inflammation have been described, indicating their potential in preventing loss of proteostatic control and formation of AF substrates.\textsuperscript{48,84-86} It is unclear if HSPs could affect the formation of triggers. However, since triggers need a vulnerable substrate to act on\textsuperscript{22}, the prevention of AF substrate formation might be sufficient to protect against first-onset AF. Indeed in various models for first-onset AF, HSPs provide protection against AF-substrate formation and hence AF initiation. In a canine model (acute) atrial...
ischemia related AF, GGA pretreatment induced HSPA1A expression and revealed protection against prolongation of effective refractory period (ERP) and atrial conduction abnormalities, thereby preventing AF initiation. These observations suggest that HSP induction may protect against some forms of AF in patients with coronary artery disease. Furthermore, a recent study in rats showed that induction of HSPA1A prevents both the Angiotensin II mediated atrial fibrosis and increased atrial vulnerability for AF induction. The findings suggest HSPA1A to play a role in inhibiting the development of a non-cardiomyocyte substrate for AF induction. In two clinical studies, HSPA1A has been implicated as cardioprotective, showing a correlation between HSPA1A atrial expression levels and reduced incidence of post-operative AF in patients in sinus rhythm undergoing cardiac surgery.

In addition to HSPA1A, other HSPs might be involved in primary prevention of AF. In patients with AF, increased mitochondrial HSP expression levels, i.e. HSPD1, HSPE1 and mortalin (HSPA9B) have been reported. In addition, increased HSPD1 antibody levels in the serum of patients have been associated with the occurrence of post-operative AF, suggesting HSPD1 as a marker for mitochondrial and cardiac damage and subsequent increased risk for AF. Increased expression of mitochondrial chaperones could contribute to an increased protection against oxidative stress. Therefore, these HSP family members might contribute to survival of cardiomyocytes by maintaining mitochondrial integrity and capacity for ATP generation. To date, however, several studies showed opposing correlations between the expression of mitochondrial HSPs and AF, thereby obscuring their involvement in protection against AF.

5. HSPs in the prevention of AF progression and recurrence (secondary prevention)

Various in vitro and in vivo models for tachypacing-induced AF progression identified HSPs to protect against the derailment of proteostasis and cardiomyocyte remodeling. In tachypaced HL-1 atrial cardiomyocytes for AF, a general HSP induction via a mild heat shock or by a HSP-inducing drug geranylgeranylacetone (GGA), conserved cardiomyocyte proteostasis during tachypacing and protected against subsequent electrical, contractile and structural remodeling. Furthermore, in canine models for AF progression, GGA pretreatment induced HSP (HSPA1A and HSPB1) expression and revealed protective effects against shortening of ERP, shortening of APD, reductions in L-type Ca\(^{2+}\) current and AF progression. Also, in clinical studies, a potent HSR and high HSPB1 levels have been associated with restoration of normal sinus rhythm in patients with permanent AF after mitral valve surgery. Two other studies comparing paroxysmal versus persistent AF and sinus rhythm, found an inverse correlation between HSPB1 atrial expression and AF duration and extent of myolysis. Suggesting, a temporary activation of the HSR during
a short duration of AF but exhaustion in time related to the duration of AF. Consequently, cardiomyocytes lose the ability for proteostatic control, inducing remodeling, which will result in AF progression and recurrence.

Further studies investigated the role of individual HSPs in protection against tachypacing-induced remodeling. HSPB1, and not HSPA1A, was found to play an important role, as its exclusive overexpression appears sufficient to protect against tachypacing-induced remodeling, comparable to GGA pre-treatment.\textsuperscript{15,46} Conversely, the protective effect of a general HSR or GGA pre-treatment on tachypacing-induced changes was annihilated by a selective knockdown of HSPB1. However, in addition to HSPB1, also other HSPB family members (HSPB6, HSPB7 and HSPB8) protect against AF-induced structural remodeling independently from HSPB1.\textsuperscript{48} Hence, multiple HSPB family members prevent against AF-induced cardiomyocyte remodeling and AF progression by preserving cell proteostasis, thereby demonstrating their therapeutic potential in AF.

Taken together, there seems to be a strong case for induction of HSPs to prevent AF initiation, recurrences and progression, by attenuation of electrical, contractile and structural cardiomyocyte remodeling. There are strong indications that this effect is via normalization of cell proteostasis.

6. Mode of action for HSPs to normalize proteostasis

It has been recognized that HSPs protect against derailment of proteostasis by preventing cardiomyocyte remodeling at different stages (Figure 1 and 2). The exact mechanisms in prevention of AF initiation (primary prevention) and recurrences (secondary prevention) are not known, but are likely due to HSP regulated protection against various AF promoting factors that induce the substrate for AF initiation and progression.

6.1. Ion channel currents

An ion channel current is dependent on the expression level, phosphorylation and redox status of the channel\textsuperscript{29,58,93}, as well as the stability of the cytoskeleton\textsuperscript{94} and Rho-GTPase activity.\textsuperscript{95} The HSP-inducing drug GGA previously showed protective effects against tachypacing-induced reductions in L-type Ca\textsuperscript{2+} current and shortening of APD.\textsuperscript{46} Furthermore, several studies have shown protective effect of HSPs on almost all of these regulating factors. HSPs are known to interact and, in some cases, inhibit kinases and phosphatases, who’s activity is altered during AF\textsuperscript{83,96-100}, thereby potentially preventing or normalizing the phosphorylation status of ion channels, especially L-type Ca\textsuperscript{2+} channel.\textsuperscript{61} Furthermore, several HSPs (including HSPB1) were shown to provide protection against oxidative stress, thereby potentially preventing or restoring the redox status of the ion channels.\textsuperscript{101} If HSPs can influence the expression levels of ion channels is currently not
known. Lastly, also the stability of the actin cytoskeleton and Rho-GTPase activity are regulated by the small HSP family members (see below). The findings reveal a protective role of HSP against AF-induced changes in ion channel current, including reductions in the L-type Ca\(^{2+}\) current.

### 6.2. Kinome activity and target proteins

Various kinases and phosphatases reveal changed activity during AF, which contributes to cardiomyocyte remodeling depending on their target proteins. In addition to ion-channels, known targets are transcription factors, various calcium handling proteins and the actin cytoskeleton. Changes in transcription factor phosphorylation, regulates gene expression and hence can induce an altered gene expression profile, possibly contributing to cardiomyocyte hibernation. Interestingly, HSPB1 was shown to interact with certain (downstream) kinases, such as IkappaB kinase and c-Jun N-terminal kinase (JNK), thereby suppressing activation of the transcription factor NF-kappaB. Interestingly, these kinases have also been found to be modulated during AF. In addition, HSPB1 is known to interact with other kinases and phosphatases and thereby might prevent the activation of other downstream transcription factors.

Changes in phosphorylation status of calcium handling proteins will affect the calcium homeostasis in cardiomyocytes. It is generally accepted that AF-induced abnormalities in intracellular Ca\(^{2+}\) handling leads to atrial cardiomyocyte stress and induces remodeling that contributes to the progression of AF. A calcium overload can be caused by an increase in L-type Ca\(^{2+}\) channel activity, or a changed activity of calcium handling proteins such as RyR2, SR Ca\(^{2+}\) ATPases or Na\(^{+}\)/Ca\(^{2+}\) exchanger. These rapid changes in activity of proteins involved in calcium handling are modulated by kinases and/or phosphatases, including CAMKII and PP1, of which the activities are increased during AF. Interestingly, studies showed that HSPs interact with CaMKII, calcineurin and PP1. Furthermore, HSPB6 was shown to inhibit PP1 activity. Also, HSPs increase SR Ca\(^{2+}\) ATPase activity and stimulate both the reuptake of Ca\(^{2+}\) into the SR and the extrusion of Ca\(^{2+}\) out of the cardiomyocyte via Na\(^{+}\)/Ca\(^{2+}\) exchanger. These findings suggest that HSPs can protect against (tachypacing-induced) changes in calcium handling proteins, resulting in attenuation of AF progression.

AF is known to activate RhoA-GTPase and ROCK and induces subsequent F-actin stress fiber formation contributing to contractile dysfunction. Several HSPB family members (HSPB1, HSPB6 and HSPB7) were recently shown to bind to actin and prevent F-actin stress fiber formation downstream of RhoA/ROCK. HSPB1 and HSPB6 even promoted actin stress fiber disassembly. HSPB8 did not directly bind actin, but instead inhibited upstream RhoA GTPase activation, thereby preventing F-actin stress fiber
formation. As most HSPB family members are known to protect the actin cytoskeleton from remodeling, this action likely represents an important mechanism by which HSPBs attenuate AF-induced derailment of proteostasis and cardiomyocyte remodeling.

6.3. HSPs and oxidative stress

Interestingly, in AF patients, an increase in oxidative stress markers has been observed and anti-inflammatory or anti-oxidant treatment with glucocorticoids and statins suppressed atrial remodeling and have shown some clinical value in prevention of post-surgery AF (primary prevention), substantiating a role for oxidative stress in AF-induced remodeling. Glucocorticoids and statins have been reported to induce several HSPs (HSPB1, HSPB5 and HSPA1A), leaving open the possibility that part of their protective pleiotropic effects is due to overexpression of HSPs. HSP induction can provide protection against oxidative stress by several mechanisms. HSPB1 is known to regulate the redox status of cardiomyocytes by maintaining glutathione in its reduced form, thus decreasing the amount of reactive oxygen species (ROS) produced in cells exposed to oxidative stress or tumor necrosis factor TNFalpha. HSPB1 may therefore prevent tachypacing-induced alterations in redox status of cardiomyocytes and thereby preserve cell proteostasis and electrophysiological and contractile function of the cardiomyocyte in AF. In addition to alterations in redox state, oxidative stress can also contribute to actin cytoskeleton instability, resulting in impairment of cardiomyocyte contractile function. Several members of the HSPB-family were found to bind the actin filaments and prevent their disruption in response to various stresses, including AF.

7. Therapeutic potential of HSP inducing drugs in AF

Pharmacological approaches preventing the substrate for AF are being studied, with the hope that they might be useful therapeutic agents in treating AF. So far, the efficacy of commonly used drugs, including glucocorticoids and statins, on remodeling is limited and (serious) adverse effects have been reported, indicating the need for more effective therapeutic agents with less adverse effects. Since derailment of cellular proteostasis results in cardiomyocyte remodeling and AF progression and derailment is counteracted by HSPs, pharmacological induction of the HSR seems to represent a key target for upstream therapy.

Currently, GGA represents the most promising compound for the pharmacological induction of HSPs. Until now it is the most efficacious HSP boosting drug. Furthermore, in contrast to other HSP inducers, GGA is a nontoxic compound shown to be capable of inducing HSP expression in various tissues, including gastric mucosa, intestine, liver, myocardium, retina, kidney, and central nervous system. In addition GGA is used clinically in Japan since 1984 as an antiulcer drug and no serious adverse reactions have been
reported.\textsuperscript{124-127} GGA rapidly induces HSP expression (HSPB, HSPA1A, HSPC family members) via activation of the heat shock transcription factor HSF1, in response to a variety of stresses, whereas its effect is weaker under non-stress conditions, providing its main effect when and where needed.\textsuperscript{128,129}

The protective effect of GGA-induced HSP expression on early and late remodeling, suggests that it has value in the prevention of clinical AF, although this still needs to be assessed in clinical trials.\textsuperscript{46,87,130} So far, the protective action of GGA has been established regarding electrical, contractile and structural remodeling in \textit{in vitro} HL-1 and dog atrial cardiomyocytes and in \textit{in vivo} dog models for AF. GGA also has beneficial effects in AF of different origin, as observed in AF induced by congestive heart failure and acute ischemia.\textsuperscript{87,116} The broad protective effects of GGA against AF-related derailment of proteostasis and atrial remodeling suggest that inducers of the HSR have substantial therapeutic value for clinical AF. Other drugs that induce HSP expression, such as bimoclomol, atorvastatin, cyclosporine A, dexamethasone, still need to be tested for their protective effects against AF-induced remodeling. Nevertheless, their therapeutic potential in other cardiac diseases, such as ischemic heart disease, have already been documented.\textsuperscript{74,131-135}

In summary, AF results in a derailment of cardiomyocyte proteostasis by inducing reversible electrical and irreversible structural remodeling. There is strong evidence that induction of HSPs, in particular HSPB family members, normalizes proteostasis and thereby prevents electrical and structural remodeling. Known upstream targets for HSP protection include L-type Ca\textsuperscript{2+} channel, calcium handling proteins, calpain, RhoA-GTPase and F-actin stress fibers. Ultimately, the induction of HSPs, by proteostasis regulators such as GGA, may prevent the occurrence of AF (primary prevention) and may contribute to enhance therapeutic efficacy and treatment options for patient with AF in delaying progression towards persistent AF and/or improve the outcome of cardioversion (secondary prevention).

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