Cardioversion of persistent atrial fibrillation by a combination of atrial specific and non-specific class III drugs in the goat

Y. Blaauw, U. Schotten, A. van Hunnik, H.R. Neuberger, M.A. Allessie*

Department of Physiology, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, The Netherlands

Received 16 January 2007; received in revised form 19 March 2007; accepted 25 March 2007

Available online 30 March 2007

Time for primary review 20 days

Abstract

Objective: In electrically remodeled atria the effect of blockers of the delayed rectifier K+ current I Kr on repolarization is reduced, whereas the efficacy of ‘early’ class III drugs (I Kur/Ito/I Kach blockers) is enhanced. We evaluated the electrophysiological and antifibrillatory effects of AVE0118, dofetilide, and ibutilide (alone and in combination) on persistent atrial fibrillation (AF) in the goat.

Methods and results: The effects of separate and combined administration of AVE0118, dofetilide, and ibutilide were determined before and after 48 h of AF. AVE0118 alone markedly prolonged the atrial refractory period (400 ms cycle length) (AERP400) before and after 48 h of AF. The prolongation of AERP400 by dofetilide and ibutilide, respectively, was reduced by AF from 22±2 to 7±2 ms (p<0.01) and 25±5 to 5±2 ms (p=0.01). Pre-treatment with AVE0118 restored the prolongation of AERP400 by dofetilide or ibutilide (to 20±3 and 30±6 ms; p<0.01). This effect was atrial specific since the QT-interval was not changed. The antifibrillatory action was evaluated in 10 goats that were in persistent AF for 57±7 days. Dofetilide (20 μg/kg/h) or ibutilide (4 mg/h) alone restored sinus rhythm in only 20% of the animals. AVE0118 (1, 3, and 10 μg/kg/h) terminated AF in 11, 30, and 60%, respectively. Additional infusion of IKr blockers caused an additional number of cardioversions, resulting in a final cardioversion rate of 56, 80, and 100%, respectively. AVE0118 alone prolonged the AF cycle length (AFCL) while the conduction velocity during AF (CVAF) remained unchanged (70 ±1 vs. 68 ±2 cm/s; p=0.3). Addition of dofetilide or ibutilide caused a synergistic increase in AFCL and a slight increase in CVAF to 74±1 cm/s (p<0.001). The length of the reentrant trajectories increased from 7.6±0.3 (control) to 11.6±0.5 cm after AVE0118 alone (p<0.001) and 14.8±0.8 cm after addition of dofetilide or ibutilide (p<0.001).

Conclusions: In electrically remodeled atria, blockade of I Kur/Ito/I Kach restored the class III action of IKr blockers. Persistent AF could be effectively cardioverted by infusion of a combination of AVE0118 and dofetilide or ibutilide. This antifibrillatory action was associated with an almost twofold lengthening of the intra-atrial pathways for reentry.

© 2007 European Society of Cardiology. Published by Elsevier B.V. All rights reserved.

Keywords: antiarrhythmia agents; electrophysiology; atrial fibrillation; K-channel

1. Introduction

Class III drugs, currently used for the treatment of atrial fibrillation (AF), exert their antifibrillatory action mainly by blockade of the rapid component of the delayed rectifier I Kr [1]. Unfortunately, the cardioversion success rate rapidly decreases with the duration of AF [2]. This loss of efficacy can partly be explained by atrial electrical remodeling during the first days of AF. In patients as well as in animals, electrical remodeling has been shown to reduce the effects of sotalol, dofetilide, or ibutilide on prolongation of the atrial refractory period [3–5]. Due to AF-induced down-regulation of the L-type Ca2+-current [6], the action potential duration shortens. As a result, the relative contribution of I Kr
decreases and repolarization is now mainly carried by earlier activated K’-currents like I_{to}, I_{Kur} and I_{K,Ach} [5,7,8].

AVE0118 is a novel atrial specific class III drug that blocks the early activated K’-currents I_{Kur}, I_{to} and I_{K,Ach} (see Gogelein et al. for differences in relative potencies) [9]. In contrast to I_{Kr}-blockers, the atrial class III action of this compound has recently been shown not to be reduced by AF. Actually, in remodeled atria the effects on the atrial action potential and refractory period were enhanced [5,8]. In the goat, the success rate of AVE0118 to cardiovert persistent AF of 53±19 days was 63% [5]. The ventricles were not affected since the QT-interval remained unchanged (atrial specific class III drugs) [5].

In the present study we evaluated whether the cardioversion success rate of AVE0118 could be further increased by combining it with an I_{Kr}-blocker. We hypothesized that restoration of the plateau phase of the remodeled action potential by AVE0118 would restore the original contribution of I_{Kr} to atrial repolarization. This in turn would reinstitute the original antiarrhythmic action of I_{Kr}-blockers. Such a synergistic action between ‘early’ and ‘late’ class III drugs was expected to exert still only a limited effect on the ventricles (QT-time). The potential value of such a drug combination would be that AF can be effectively cardioverted without the risk of ventricular proarrhythmia.

2. Methods

2.1. The Goat Model of AF

Fourteen female goats weighing 53±2 kg were used. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). During general anesthesia, four teflon felt plaques with a total of 117 unipolar electrodes were sutured on Bachmann’s bundle (n=54), the free wall of the right and left atria (30 each), and the left ventricle (n=3). The leads were tunneled subcutaneously and exteriorized at the neck [10]. The study protocol was started 3–4 weeks after surgery. Atrial fibrillation was maintained by a fibrillation pacemaker, that automatically delivered a burst of rapid stimuli (50 Hz, 1 s), immediately after spontaneous termination of AF [11].

2.2. Electrophysiological Measurements

Measurements were performed in the awake state. The atrial effective refractory period (AERP) was determined at the free wall of the right and left atrium during regular pacing (2 ms duration; 4× threshold) with intervals between 400 and 200 ms. Single interpolated stimuli were applied after every 8th basic stimulus and the coupling interval was prolonged in 2 ms increments. The longest coupling interval that failed to capture the atria was taken as the AERP. Inducibility of AF was evaluated by applying single premature stimuli at the right and left atrial free wall during regular pacing with an interval of 400 ms. AF was considered inducible if this resulted in AF paroxysms of more than 1 s duration. Atrial conduction velocity was measured along Bachmann’s bundle at two pacing cycle lengths (400 and 250 ms).

The median AF cycle length (AFCL) was calculated from at least 300 consecutive AF cycles recorded from the right or the left atrium. Conduction velocity during persistent AF was measured from the electrode array on the right or left atrium, as described recently [12]. Briefly, all conduction vectors within areas of 3×3 electrodes (8×8 mm) were calculated from at least 6 local activation times. This resulted in a histogram of a large number of local conduction velocities (N:1004±188; range 198–2017). The median velocity was taken as the dominant conduction velocity during AF (see Houben et al. [12] for a more detailed description).

The QT-time duration was measured from a precordial electrocardiogram or a unipolar left ventricular electrogram during atrial pacing (cycle length of 400 ms). To evaluate the effects of class III drugs on the action potential, monophasic action potentials were recorded from the right atrium in 3 goats using a Franz catheter (general anesthesia).

2.3. Experimental protocol

In each goat different experiments, with multiple drugs were performed in both non-remodeled and remodeled atria and also during persistent AF. The separate and combined effects of AVE0118 and dofetilide (n=6) and AVE0118 and ibutilide (n=7) on atrial and ventricular electrophysiology were studied before and after 1–4 days (median 2 days) of AF-induced electrical remodeling. First, AVE0118 was administered intravenously during 1 h at a constant rate of 3 μg/kg/h. Then, while infusion of AVE0118 was continued, either dofetilide (20 μg/kg/h) or ibutilide (0.12 μg/kg/h) was started. Electrophysiological measurements were performed during control, after 30 min of AVE0118 alone, and after 30 min of the combination. The effects of dofetilide and ibutilide alone were measured in separate experiments (after 30 min infusion). The different drugs were studied in random order with at least 2 days between experiments.

The effects of different dosages of AVE0118 (0.3, 1, 3 or 10 μg/kg/h) in combination with dofetilide (20 μg/kg/h) or ibutilide (4 mg/h) on persistent AF were measured in 10 goats (AVE0118+dofetilide (n=5); AVE0118+ibutilide (n=5). On different days, in each goat a certain dosage of AVE0118 (including saline alone) was infused during 1 h, followed by a fixed dosage of either dofetilide or ibutilide. The effects on AFCL were continuously monitored and in case of cardioversion, AF was reinduced by burst pacing to evaluate the reproducibility of cardioversion.

Experiments were performed in a paired design, i.e. in each goat that received the combination of AVE0118 and an I_{Kr}-blocker also the effect of the same I_{Kr}-blocker alone was evaluated.
2.4. Statistical analysis

Differences between groups were evaluated by the paired Student’s t-test or by 2-way repeated ANOVA with post hoc Bonferroni’s t-test. A chi-square test was used to compare the inducibility of AF. Differences were considered statistically significant at a P-value of <0.05. Results are presented as mean±SEM.

3. Results

3.1. Electrophysiological Effects of AVE0118, Dofetilide and Ibutilide

Fig. 1 shows the separate and combined effects of AVE0118, dofetilide and ibutilide on the atrial refractory period both before and after 48 h of AF. In non-remodeled atria

### Table 1

Separate and combined effects of AVE0118, dofetilide and ibutilide on atrial conduction velocity and inducibility of AF

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Dofetilide</th>
<th>Control</th>
<th>AVE0118</th>
<th>AVE0118+Dofetilide</th>
<th>Control</th>
<th>Ibutilide</th>
<th>Control</th>
<th>AVE0118</th>
<th>AVE0118+Ibutilide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non remodeled atria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CV400 (cm/s)</td>
<td>121±7</td>
<td>120±6</td>
<td>120±7</td>
<td>117±7</td>
<td>115±6**</td>
<td>130±5</td>
<td>128±5</td>
<td>126±6</td>
<td>123±7</td>
<td>120±5**</td>
</tr>
<tr>
<td>CV250 (cm/s)</td>
<td>115±7</td>
<td>114±6</td>
<td>113±7</td>
<td>106±6</td>
<td>99±4**</td>
<td>119±6</td>
<td>113±6*</td>
<td>120±5</td>
<td>114±5*</td>
<td>106±5**†</td>
</tr>
<tr>
<td>Ind AF (%)</td>
<td>17% (2/12)</td>
<td>8% (1/12)</td>
<td>0% (0/12)</td>
<td>0% (0/12)</td>
<td>8% (1/12)</td>
<td>7% (1/14)</td>
<td>7% (1/14)</td>
<td>21% (3/14)</td>
<td>0% (0/14)</td>
<td>0% (0/14)</td>
</tr>
</tbody>
</table>

|                  |         |            |         |         |                   |         |           |         |         |                   |
| Remodeled atria  |         |            |         |         |                   |         |           |         |         |                   |
| CV400 (cm/s)     | 124±6   | 123±7      | 125±8   | 123±7   | 127±8             | 128±8   | 126±6     | 124±6   | 119±6**††    |
| CV250 (cm/s)     | 118±6   | 118±5      | 121±8   | 114±6   | 110±6*            | 120±5   | 115±6     | 121±6   | 115±6*  | 110±5**††    |
| Ind AF400 ms     | 100%    | 100%       | 100%    | 33%     | 8%                | 100%    | 100%      | 100%    | 31%     | 23%              |
| (no of sites)    | (12/12) | (12/12)    | (12/12) | (4/12)** | (1/12)**          | (13/13) | (13/13)   | (13/13) | (4/13)** | (3/13)**        |

*p<0.05 versus control, ** p<0.01 versus control, †p<0.05 versus AVE0118, ††p<0.01 versus AVE0118.
(upper panels) all drugs prolonged the AERP in a reverse frequency-dependent manner. AVE0118 and dofetilide alone increased the AERP400 by respectively 51±5 and 22±2 ms and given together by 75±6 ms. The combined effect was not different from the sum of the separate effects (73±7 ms; \( p=0.3 \)). A similar additive effect was observed for AVE0118 and ibutilide (separate effects 44±2 and 25±5 ms; combined effect 72±7 ms). After 48 h of AF (lower panels) the AERP had become markedly abbreviated. The action of dofetilide was now significantly reduced (prolongation of AERP400 7±2 ms; \( p<0.01 \) vs. non-remodeled atria). In contrast, AVE0118 exerted a stronger class III action (72±5 ms; \( p=0.01 \)). After 48 h of AF, the combination of AVE0118 and dofetilide prolonged the AERP400 by 92±6 ms, which was significantly more than the sum of their separate effects (79±5 ms; \( p<0.01 \)). This was especially true at longer pacing intervals when the action of dofetilide was most severely reduced by electrical remodeling. During pacing at 200 ms cycle length, the effect of dofetilide was identical before and after AVE0118.

Similar results were observed with the combination of AVE0118 and ibutilide. The combined administration of AVE0118 and ibutilide prolonged the AERP400 with 94±92 ms.

Fig. 2. Effect of separate and combined effect of AVE0118 (3 \( \mu \)g/kg/h) and dofetilide (20 \( \mu \)g/kg/h) and ibutilide (0.12 \( \mu \)g/kg/h) on the QT-duration measured during atrial pacing (cycle length 400 ms). Experiments were performed before and after 48 h of AF. The bargraphs represent the mean results of 6 (dofetilide) and 7 animals (ibutilide). *\( p<0.05 \), **\( p<0.01 \).

Fig. 3. Example of the effects of separate and combined administration of dofetilide (20 \( \mu \)g/kg/h) and AVE0118 (3 \( \mu \)g/kg/h) on the AFCL. Shown is an unipolar electrogram recorded from the right atrium. Dofetilide alone (5 \( \mu \)g/kg; cumulative dosage after 15 min infusion) slightly prolonged the median AFCL from 98 to 110 ms, whereas AVE0118 (3 mg/kg; cumulative dosage after 1 h) increased the AFCL to 140 ms. After the combined administration of dofetilide (cumulative dosage 5 \( \mu \)g/kg) and AVE0118 (cumulative dosage 3.75 mg/kg) the AFCL was longer (175 ms) than expected from the sum of the separate effects (synergism).
9 ms compared to the sum of their separate effects of 68±6 ms ($p<0.01$). Thus, whereas in non-remodeled atria the class III effects of AVE0118 and $I_{Kr}$-blockers were additive, after electrical remodeling the effects of AVE0118 and $I_{Kr}$-blockers have become synergistic.

Table 1 shows the effects on atrial conduction velocity. During pacing at a cycle length of 400 ms, conduction velocity was only slightly decreased by AVE0118 or a combination with dofetilide or ibutilide. During rapid pacing (250 ms) a more pronounced slowing of conduction was observed, both in normal and remodeled atria. This effect on conduction velocity can be explained by incomplete recovery of excitability due to prolongation of AERP.

The effects on QT-duration are shown in Fig. 2. Dofetilide and ibutilide significantly prolonged the QT-interval, whereas AVE0118 had no effect. The combination of AVE0118 and dofetilide or ibutilide prolonged the QT-time to the same extent as dofetilide or ibutilide alone. This was true both before and after 48h of AF. Thus, in contrast to the effects on atrial refractoriness, the effect of $I_{Kr}$-blockers on QT-time was not enhanced by AVE0118.

### 3.2 Inducibility of AF

The inducibility of AF was determined before and after 48 h of electrical remodeling (Table 1). In non-remodeled atria, single premature stimuli induced AF in 0–21% of the cases. In contrast, after 48h of AF, paroxysms of AF were induced in 100%. After dofetilide and ibutilide inducibility was still 100%. Infusion of AVE0118 reduced the inducibility of AF to 31–33%. After the combination of AVE0118 and dofetilide or ibutilide atrial vulnerability was respectively 8% (AVE0118+dofetilide) and 23% (AVE0118+ibutilide) of the cases. This effect was not statistically significant different from AVE0118 alone.

### 3.3 Effects on persistent AF

The separate and combined effects of AVE0118 and dofetilide or ibutilide on persistent AF (duration 57±7 days) were studied in 10 goats. Fig. 3 shows a representative example. During control, the median AFCL was 98 ms. Administration of dofetilide (5 μg/kg) or AVE0118 (3 mg/kg)

![Fig. 4](image-url)

Fig. 4. Left: AFCL during 1 h infusion of saline or AVE0118 (0.3, 1, 3 and 10 μg/kg/h) followed by additional administration of dofetilide (20 μg/kg/h) or ibutilide (4 mg/h). Right: increase in atrial fibrillation cycle length ($\Delta$AFCL) after infusion of dofetilide (cumulative dose of 5 μg/kg) (top) or ibutilide (cumulative dose of 1 mg) after pre-treatment with AVE0118 (different dosages). Dofetilide and ibutilide caused a stronger prolongation of the AFCL when given after infusion of the higher dosages of AVE0118 (1.3 and 10 μg/kg/h). The datapoints represent the mean results of 5 (dofetilide) and 5 animals (ibutilide). *$p<0.05$, **$p<0.01$ versus dofetilide or ibutilide alone.
prolonged the AFCL to respectively 110 and 140 ms. Given in combination, the AFCL increased to 175 ms. This prolongation by 77 ms was larger than the sum of the separate effects of AVE0118 and dofetilide (+54 ms). This synergistic action of early and late potassium blockers on AF is shown in more detail in Fig. 4. Infusion of AVE0118 increased the AFCL in a dose dependent manner. The amount of prolongation of the AFCL by dofetilide and ibutilide was dependent on the pretreatment with AVE0118. This is quantified in the right panels of Fig. 4. While given alone, dofetilide and ibutilide prolonged the AFCL by only 11±3 and 12±3 ms, after increasing dosages of AVE0118 their class III effect was clearly enhanced (AAFC: AVE0118 1 μg/kg/h: 16±1 (p=0.02) and 33±7 ms (p=0.08) respectively dofetilide and ibutilide, AVE0118 3 μg/kg/h: 33±6 (p=0.01) and 37±4 ms (p<0.01) and AVE0118 10 μg/kg/h: 32±7 (p=0.03) and 37±4 ms (p<0.01). Pre-treatment with AVE0118 (3 and 10 μg/kg/h) during 1 h, thus enhanced the effects of dofetilide or ibutilide on AFCL up to as much as threefold.

Fig. 5 shows the success rate of cardioversion (grouped data). Infusion of AVE0118 terminated AF in a dose dependent manner (respectively 11% (1 μg/kg/h), 30% (3 μg/kg/h), and 60% (10 μg/kg/h)). Administration of dofetilide (20 μg/kg/h) or ibutilide (4 mg/h) alone had a success rate of only 20%, both 1 of 5 goats. After pre-treatment with increasing dosages of AVE0118, infusion of Ik,-blockers caused an additional number of cardioversions in the goats that failed to cardiovert during infusion of AVE0118 alone, resulting in a final cardioversion rate of respectively 56, 80 and 100%.

3.4. MAP recordings

The class III action of AVE0118, dofetilide and ibutilide on persistent AF was further evaluated by recording right atrial monophasic action potentials during pharmacological cardioversion (Fig. 6). During persistent AF with a median AFCL of 87 ms (upper tracing) MAPs were 'triangular' in shape without a prominent plateau phase. The lengthening in...
AFCL by AVE0118 (second tracing) was associated with a prolongation of the plateau-phase of the action potential. Addition of dofetilide (or ibutilide) caused a further MAP prolongation and lengthening of the AFCL (third tracing). Shortly before cardioversion (lower tracing), the MAP duration was markedly increased (MAP 70 217–246 ms) and the AFCL was prolonged to more than 300 ms. These recordings support the notion that prolongation of the AFCL and restoration of sinus rhythm are a direct effect of prolongation of the atrial action potential.

Fig. 7. Representative example of the effects of AVE0118 alone (cumulative dose of 3 mg/kg) and in combination with dofetilide (5 μg/kg) on the AFCL, conduction velocity and fibrillation pathlength measured during persistent AF in a single goat. AVE0118 prolonged the AFCL, did not affect the conduction velocity and markedly prolonged the pathlength of the fibrillation waves. Administration of dofetilide caused an additional prolongation of the AFCL, slightly increased conduction velocity, and increased the fibrillation pathlength.

AFCL by AVE0118 (second tracing) was associated with a prolongation of the plateau-phase of the action potential. Addition of dofetilide (or ibutilide) caused a further MAP prolongation and lengthening of the AFCL (third tracing). Shortly before cardioversion (lower tracing), the MAP duration was markedly increased (MAP 70 217–246 ms) and the AFCL was prolonged to more than 300 ms. These recordings support the notion that prolongation of the AFCL and restoration of sinus rhythm are a direct effect of prolongation of the atrial action potential.

Fig. 8. Effects of AVE0118 alone and in combination with the IKr-blockers dofetilide and ibutilide, on AF cycle length, conduction velocity and the estimated pathlength of the fibrillation waves. Measurements were performed during control, 30 and 60 min after infusion of AVE0118 (3 μg/kg/h) and after additional infusion of dofetilide (cumulative dose of 5 μg/kg; n = 5 goats, 8 sites) or ibutilide (cumulative dose 1 mg; n = 3 goats, 5 sites). Shown is pooled data. AVE0118 significantly prolonged the AFCL, did not affect conduction velocity and increased the fibrillation pathlength. The additional infusion of an IKr-blocker caused a further prolongation of the AFCL and a slight increase in conduction velocity. **p < 0.001 vs. control, ††p < 0.001 versus AVE0118.

Fig. 8. Effects of AVE0118 alone and in combination with the IKr-blockers dofetilide and ibutilide, on AF cycle length, conduction velocity and the estimated pathlength of the fibrillation waves. Measurements were performed during control, 30 and 60 min after infusion of AVE0118 (3 μg/kg/h) and after additional infusion of dofetilide (cumulative dose of 5 μg/kg; n = 5 goats, 8 sites) or ibutilide (cumulative dose 1 mg; n = 3 goats, 5 sites). Shown is pooled data. AVE0118 significantly prolonged the AFCL, did not affect conduction velocity and increased the fibrillation pathlength. The additional infusion of an IKr-blocker caused a further prolongation of the AFCL and a slight increase in conduction velocity. **p < 0.001 vs. control, ††p < 0.001 versus AVE0118.
3.5. Prolongation of the pathlength of fibrillation waves

To evaluate whether class III drugs terminated AF by lengthening of the trajectory of the fibrillation waves, we calculated changes in the pathlength of the fibrillation waves (PLAF). Changes in individual pathlength of the fibrillation waves were estimated by multiplying their measured conduction velocities and cycle lengths (PLAF = CVAF × AFCL), both during control and after administration of AVE0118 (3 μg/kg/h) and dofetilide or ibutilide. Fig. 7 shows an example of changes in AFCL, CVAF and PLAF by administration of class III drugs during persistent AF (46 days). During control, the AFCL histogram showed a normal distribution with a median cycle length of 111 ms. The median CVAF was 76 cm/s and the pathlength was 8.3 cm. Administration of AVE0118 prolonged the AFCL to 154 ms. Because the conduction velocity remained the same (74 cm/s), the median PLAF prolonged to 11.1 cm. In this case, infusion of AVE0118 alone did not cardiovert AF and the additional infusion of dofetilide (5 μg/kg) was necessary to restore sinus rhythm. The AF histograms just prior to cardioversion are shown at the bottom. The combination of early and late class III drugs prolonged the AFCL to as much as 203 ms. Since conduction velocity was still not affected or even slightly increased (79 cm/s), this further increase in AFCL led to a marked lengthening of the PLAF to 16.5 cm.

In Fig. 8 the effects on AFCL, CVAF and PLAF on persistent AF are shown for all goats. Administration of AVE0118 alone (60 min infusion) prolonged the AFCL from 109±3 to 167±6 ms (p<0.001) while CVAF remained unchanged (70±1 to 68±2 cm/s; p=0.3). The addition of dofetilide or ibutilide further prolonged the AFCL to 198±9 ms (p<0.001 vs. AVE0118) and slightly increased the CVAF to 74±1 cm/s (p<0.001 vs. AVE0118). As a result the PLAF increased from 7.6±0.3 to 11.6±0.5 cm by AVE0118 alone (p<0.001) and to 14.8±0.8 cm by the combination of AVE0118 with dofetilide or ibutilide (p<0.001 vs. AVE0118). Thus, the estimated length of the reentrant pathways during persistent AF was prolonged almost twofold by administration of a combination of early and late class III drugs.

4. Discussion

4.1. Synergistic action of AVE0118 and IKr-blockers

Recent experimental and clinical studies demonstrated that the efficacy of IKr-blockers is diminished after AF-induced electrical remodeling [3,4]. In contrast, blockade of IKur, Ito and IKACH by AVE0118 exerted an enhanced class III effect in electrically remodeled caprine atria [5]. A possible explanation for the reduced effect of IKr-blockers and enhanced action of IKur-blockers was given by Courtemanche et al. [7]. They showed that the remodeled triangularized and shortened action potential results in very little IKr activation. Activation of IKr by the raised plateau, resulting from IKur inhibition tends to minimize APD prolongation by IKur-block in the normal atrium, but in the remodelled triangular action potential the IKr influence is reduced and IKur block more readily increases APD.

In the present study we found that AVE0118 could completely restore the loss of action of IKr-blockers in electrically remodeled atria. The combination of AVE0118 with dofetilide or ibutilide, thus exerted a larger effect on atrial refractoriness than the sum of their separate effects (synergism). The class III action of dofetilide and ibutilide on AERP was increased 3-fold by pre-treatment with AVE0118 (3 μg/kg/h). These data are in agreement with computer simulations of remodeled atrial myocytes, showing that simultaneous inhibition of IKur and IKr resulted in a synergistic prolongation of the action potential [7]. Also in isolated human atrial cells the combination of IKur and IKr-blockade prolonged the action potential more than expected from their individual effects [8]. All these studies support the hypothesis that restoration of the plateau phase in electrically remodeled atrium by blockade of the early activated K+-currents (IKur and Ito) restores the normal contribution of the later activated IKr to atrial repolarization. This may explain the observed synergism between ‘early’ and ‘late’ class III drugs on refractory period and arrhythmias in electrically remodeled atria.

Also during persistent AF, combined administration of AVE0118 and IKr-blockers prolonged the AFCL to a larger extent than expected from their separate effects. It cannot be excluded that apart from a strong class III effect on the action potential duration, also a widening of the temporal excitable gap contributed to this strong prolongation in AFCL. Due to prolongation of the action potential the number of fibrillation waves decrease, and the size of the functional circuits increase. As a consequence, the waiting time for reexcitation (temporal excitable gap) will become longer.

4.2. Antifibrillatory mechanism of the combination of AVE0118 and IKr-blockers

In the present study we measured the effects of AVE0118, dofetilide and ibutilide on the AFCL and conduction velocity of the fibrillation waves, to estimate the effects of these different class III drugs on the length of the infra-atrial trajectories of the waves. Pharmacological cardioversion of persistent AF by AVE0118, either alone or in combination with dofetilide or ibutilide, was preceded by a marked prolongation of the AFCL, while the CVAF remained unchanged. The combined administration of AVE0118 and an IKr-blocker, almost doubled the average pathlength of the fibrillation waves. This not only demonstrates a very powerful and effective class III effect during persistent AF, but it also offers a logical explanation for termination of the arrhythmia. Lengthening of the required infra-atrial pathways for reentry will allow less fibrillation waves to be present simultaneously in the available atrial tissue mass. When the number of fibrillation waves is reduced below a certain critical value, the chance that all fibrillation waves will die out at the same time becomes so high that AF can no longer perpetuate itself. In case AF is
perpetuated by the presence of a rapidly firing focus, the class III effect could reduce the frequency of the focus and/or lead to some degree of exit block between the focus and the atria. This would reduce the effective frequency with which the focus is driving the atria. When this driving frequency is reduced enough, at some moment all parts of the atria will be able to follow the focus in a 1:1 manner and AF will change into a regular atrial tachycardia.

4.3. Cardioversion of AF by atrial specific and non-specific class III drugs

Due to the high prevalence of AF in the elderly, pharmacological treatment remains the mainstay of AF therapy. Currently available class III drugs are only moderately effective in restoring sinus rhythm in patients with AF. During the first days of AF they terminate AF in 44–70% of the cases [2,13], but after AF has persisted for >7 days the rate of cardioversion drops to 11–51% [2,14,15]. Also the risk of ventricular pro-arrhythmia remains a matter of concern [16].

The Kv1.5 based ultrarapid delayed rectifier IKur is preferentially expressed in the atria and absent in the ventricles [17]. Hence, IKur-blockers bear the potential to suppress atrial arrhythmias without carrying the risk of ventricular pro-arrhythmia. Several of such agents are presently under development (e.g. AVE0118, RSD1235, AZD7009) [9,18,19]. In different animal models, it was shown that AF could be terminated effectively by these drugs [5,19]. In the present study we demonstrated a dose dependent cardioversion efficacy of AVE0118 ranging from 11 to 60%. Of note, in previous cardioversion experiments in the goat with the class IC agent flecainide we observed somewhat lower cardioversion success rates of 40% [10].

The results of phase II clinical trials with the agents RSD1235 and AZD7009 have recently been published [20,21]. RSD1235 terminated AF within 30 min after infusion in 56% of patients with recent onset AF (<3 days) [21]. AZD7009 restored sinus rhythm in up to 70% of patients with AF or atrial flutter with a mean duration of 43 days [20]. Both drugs were associated with a low risk of pro-arrhythmia. Pending the results of larger clinical trials, we can only speculate on the clinical efficacy and the potential impact of atrial specific antiarrhythmic drugs for the treatment of AF.

The results of the present study demonstrate that a combined blockade of IKur/Ito/IKAch and IKr offers a strong anti-arrhythmic effect. In the present goat model, this drug combination could cardiovert persistent AF in up to 100% of the animals, without excessive QT-prolongation. This suggests that such a drug combination may enhance the antiarrhythmic efficacy without increasing the risk of pro-arrhythmia.

4.4. Ventricular pro-arrhythmia

In the present study we showed that the combination of AVE0118 and dofetilide or ibutilide did not prolong the QT-interval more than the IKr-blockers alone. To our knowledge this is the first study investigating the combined effects of IKur/IKAch/Ito and IKr-blockers on QT-duration. As mentioned earlier, the contribution of IKur and IKr to ventricular repolarization is minimal. The role of Ito for the shape of the ventricular action potential is less consistent and marked differences exist between species [22]. In animals with short ventricular action potentials (like mouse and rat) blockade of the Ito markedly prolongs the duration of the action potential [23]. In larger animals, Ito only plays a role during the early phase of repolarization and Ito blockade does not affect the duration of the action potential [24]. The lack of effect of AVE0118 on the duration of the QT-interval in the goat confirms the minor role of Ito. Recently, Oros et al. evaluated the possible pro- and antiarrhythmic potential of AVE0118 in anesthetized chronic AV-block dogs [25]. Whereas after intravenous administration of dofetilide, torsades de points (TdP) arrhythmias could be induced in 8 of 8 dogs, after infusion of AVE0118, TdP arrhythmias could not be induced. Of some concern is the observation that some dogs died unexpectedly during the first 24 h after simultaneous administration of the highest dosages of AVE0118 and dofetilide [25]. In our present study we did not observe any ventricular arrhythmias or sudden death.

4.5. Study limitations

An important limitation of the present study is that no in-vitro studies were performed. For this reason, the cellular mechanisms remain speculative. In addition, there is no available literature on the expression of the different K+-channels in the caprine atrium. Extrapolating the results of the present study to humans must be done with great caution. There exist important differences in K+-currents between species [22,26]. In human atria the electrophysiological effects of AVE0118, alone or in combination with IKr-blockers, can be markedly different. Also the substrate of ‘lone’ AF in our goat model is likely to be different from the substrate in patients with persistent AF and underlying cardiovascular disease. On the other hand, notwithstanding these important limitations, the presently available data are promising. They should stimulate more extensive clinical evaluation of the effects of these drugs in patients with AF of different duration and etiology.

Acknowledgement

This study is supported by grants from the Royal Netherlands Academy of Arts and Sciences, the Dutch Heart Foundation (2005B112) and by a research grant from Sanofi-Aventis. Y.Blaauw received a fellowship from the Netherlands Organization for Scientific research (920-033-122).

Disclosures: M.Allessie and Y.Blaauw have contributed to the patent application ‘Phenylcarboxylic acid amides and IKr-channel inhibitors combination and the use for treating atrial arrhythmia’. Patent application WO 2004/082716 A1.
References


