Dietary n-3 Polyunsaturated Fatty Acids and Direct Renin Inhibition Improve Electrical Remodeling in a Model of High Human Renin Hypertension


Abstract—We compared the effect n-3 polyunsaturated fatty acids (PUFAs) with direct renin inhibition on electrophysiological remodeling in angiotensin II–induced cardiac injury. We treated double-transgenic rats expressing the human renin and angiotensinogen genes (dTGRs) from week 4 to 7 with n-3 PUFA ethyl-esters (Omacor; 25-g/kg diet) or a direct renin inhibitor (aliskiren; 3 mg/kg per day). Sprague-Dawley rats were controls. We performed electrocardiographic, magnetocardiographic, and programmed electrical stimulation. Dietary n-3 PUFAs increased the cardiac content of eicosapentaenoic and docosahexaenoic acid. At week 7, mortality in dTGRs was 31%, whereas none of the n-3 PUFA- or aliskiren-treated dTGRs died. Systolic blood pressure was modestly reduced in n-3 PUFA-treated (180±110 mm Hg) compared with dTGRs (208±33 mm Hg). Aliskiren-treated dTGRs and Sprague-Dawley rats were normotensive (110±3 and 119±6 mm Hg, respectively). Both n-3 PUFA–treated and untreated dTGRs showed cardiac hypertrophy and increased atrial natriuretic peptide levels. Prolonged QRS and QTc intervals and increased T-wave dispersion in dTGRs were reduced by n-3 PUFAs or aliskiren. Both treatments reduced arrhythmia induction from 75% in dTGRs to 17% versus 0% in Sprague-Dawley rats. Macrophage infiltration and fibrosis were reduced by n-3 PUFAs and aliskiren. Connexin 43, a mediator of intermyocyte conduction, was redistributed to the lateral cell membranes in dTGRs. n-3 PUFAs and aliskiren restored normal localization to the intercalated disks. Thus, n-3 PUFAs and aliskiren improved electrical remodeling, arrhythmia induction, and connexin 43 expression, despite a 70-mm Hg difference in blood pressure and the development of cardiac hypertrophy. (Hypertension. 2008;51[part 2]:540-546.)

Key Words: angiotensin II ■ renin inhibition ■ n-3 PUFA ■ arrhythmias ■ magnetocardiography

Hypertensive heart disease causes heart failure and arrhythmia propensity. Ischemia, cardiac hypertrophy, fibrosis, inflammation, and electrical remodeling all contribute to the pathogenesis.1,2 The renin-angiotensin-aldosterone system is a primary driver, and its blockade is state-of-the-art therapy. We provided initial evidence that direct renin inhibition (DRI) in transgenic rats harboring the human renin and angiotensinogen genes (dTGRs) improves target organ damage.3-5 Untreated dTGRs developed severe hypertension, hypertrophy, inflammation, fibrosis, and small myocardial infarctions. Ventricular arrhythmias and, consequently, sudden cardiac death contributed to the high mortality rate at the early age of 7 weeks.6 Electrical remodeling in dTGRs included dysregulation of the Ica potassium channel, Ca2+-cycling proteins, and connexin (Cx) 43 gap junctions.6,7 n-3 polyunsaturated fatty acids (PUFAs), contained in marine fish oil, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), lead to cardioprotection and reduce sudden cardiac death.8 The molecular mechanisms by which n-3 PUFAs exert their cardioprotective effects are not fully understood. n-3 PUFAs putatively affect membrane ion channels.9,10 They exert antihypertensive, antiproliferative, anti-inflammatory, and anticoagulative properties.11 In spontaneously hypertensive rats, n-3 PUFA treatment lowered blood pressure.12 The reduced vasoconstriction was mediated by inhibition of angiotensin II (Ang II)–induced intracellular Ca2+ mobilization and protein kinase C phosphorylation.13 In our dTGR model, chronic n-3 PUFA treatment lowered blood pressure and improved renal and cardiac damage.14 We now investigated the impact of n-3 PUFAs and DRI on cardiac electrophysiological remodeling in the dTGR, a model of high human renin hypertension.
Methods

Experimental Design

We studied male dTGRs (RCC Ltd) and age-matched nontransgenic Sprague-Dawley (SD) rats (Tierzucht Schönwalde).4,7,15,16 Local authorities approved the studies, and we followed American Psychological Society guidelines. Omacor, a mixture of highly concentrated n-3 PUFA-containing 46% EPA and 38% DHA-ethyl-esters, was provided by Solvay Pharmaceuticals. Novartis Pharmaceuticals provided the DRI, aliskiren. We compared untreated dTGRs (n=16), dTGR + Omacor (25-g/kg diet; n=18), dTGR + aliskiren (3 mg/kg per day by osmotic mini pump; n=9), and SD (n=10) rats. We used a higher number of untreated and Omacor-treated dTGRs, because we could not predict their mortality. Treatment was started at the age of 4 weeks. ECG, magnetocardiography (MCG), and systolic blood pressure (by tail cuff) were determined at week 7. Rats were euthanized by decapitation at the age of 7 weeks. Serum and plasma were collected for further analysis. Hearts were removed and washed with ice-cold saline, blotted dry, and weighed. In a second protocol, 7-week-old rats (dTGR: n=8; dTGR + Omacor: n=9; dTGR + aliskiren: n=8; SD: n=7) were subjected to in vivo programmed electrical stimulations.

ECG, MCG, and In Vivo Electrophysiological Studies

ECG and MCG were done under slight isoflurane anesthesia as described previously.6,7,18 Programmed electrical stimulation was performed as reported recently (please see the data supplement available at http://hyper.ahajournals.org).

Fatty Acid Composition

Left-ventricular heart tissue (n=6 per animal group) was frozen in liquid nitrogen and homogenized using a biopulverizer (Biospec Products Inc). Aliquots (10 mg) were treated with tetramethylammonium hydroxide pentahydrate, and the released free fatty acids were analyzed by liquid chromatography/mass spectrometry (FILT GmbH). Separation was performed on a Gemini column (5 mm of C18, 150×3 mm) using an ammonium acetate/acetonitrile gradient at pH 9.5. A negative single ion monitoring mode was used for the detection of the individual fatty acids. Pentadecanoic acid served as an internal standard, and quantification of individual fatty acids was performed using respective calibration curves. The same procedure was applied to determine the fatty acid composition of the different chows used for rat feeding.

Immunohistochemistry and Quantitative TaqMan RT-PCR

Ice-cold acetone-fixed cryosections (6 mm) were stained by immunofluorescence techniques, as described earlier.19 The following monoclonal antibodies were used: anti–ED-1, antifibronectin, and anticalcogen I (Southern Biotechnology), as well as polyclonal antibodies were used: anti–ED-1, antifibronectin, and anticalcogen I (Southern Biotechnology), as well as polyclonal antibodies were used: anti–ED-1, antifibronectin, and anticalcogen I (Southern Biotechnology). RNA isolation and TaqMan RT-PCR were performed as described earlier.7 We analyzed LV tissue for atrial natriuretic peptide mRNA expression. Each sample was in triplicate. The target sequences were normalized in relation to the 36B4 product. The primer sequence is available on request (Biotez).

Statistics

Data are presented as mean±SEM. Differences in mean values were tested by nonparametric Mann-Whitney exact test; arrhythmia induction was tested by χ². A value of P<0.05 was considered significant. The data were analyzed using SPSS.

Results

Untreated dTGRs, dTGR + aliskiren, and SD rats were fed standard chow, whereas Omacor-treated dTGRs received the n-3 PUFA-rich diet. This diet contained EPA and DHA in amounts representing 5.6% and 4.1% of total fatty acids, respectively (Figure 1A). Chronic n-3 PUFA treatment resulted in a marked increase of the cardiac omega-3 index (29.6±0.3% for dTGR + Omacor versus 9.8±0.6% for dTGRs, 5.9±0.2% for dTGR + aliskiren, and 6.2±0.3% for SD rats; P<0.05; Figure 1B). Cardiac EPA and DHA levels were significantly increased in Omacor-treated dTGRs compared with untreated dTGRs (1.9±0.05% versus 0.07±0.005% for EPA and 27.6±0.3% versus 9.7±0.6% for DHA, respectively; Figure 1C). The cardiac arachidonic acid (AA) levels decreased from 32.2±0.5 to 16.3±0.2 for dTGR + Omacor rats (P<0.05), indicating that AA was partially replaced by EPA and DHA. The content of all other fatty acids remained largely unaffected (Figure 1C). The cardiac fatty acid composition of SD control rats and of dTGRs treated with aliskiren was not different.
Untreated dTGRs showed 31% mortality at age 7 weeks (5 of 16; Figure 2A). In contrast, none of the Omacor and aliskiren-treated dTGRs died. Untreated dTGRs developed high systolic blood pressure, which was modestly reduced by Omacor treatment (208±5 versus 180±3 mm Hg; P<0.05; Figure 2B). In contrast, aliskiren-treated dTGRs and SD controls were normotensive (110±3 and 119±6 mm Hg, respectively). Omacor-treated and untreated dTGRs showed both increased heart weights compared with SD (1203±21 and 1213±24 mg versus 933±17 mg; P<0.05; Figure 2C). Only aliskiren prevented cardiac hypertrophy (972±13 mg). ANP expression confirmed the differences in cardiac hypertrophy between the groups. Although Omacor treated and untreated dTGRs showed high levels of ANP mRNA (24±2 and 20±1 arbitrary units), ANP expression was normalized in aliskiren-treated dTGRs (2.4±0.2 and 1.8±0.2 for SD; Figure 2D).

To analyze whether the reduced mortality might be because of lessened sudden cardiac death, we tested for arrhythmia induction. Programmed electrical stimulation showed a high nonsustained and sustained ventricular tachycardia induction rate in untreated dTGRs (75%; Figure 3A). In SD controls, the same protocol never initiated arrhythmias. The arrhythmia induction was reduced to the same extent by both treatments. Only 17% of the Omacor-treated and 17% of aliskiren-treated dTGRs responded to electrical stimulation with ventricular tachycardia (P<0.05 versus untreated dTGRs). The ventricular effective refractory period was prolonged in dTGRs compared with SD controls (54.5±1.9 versus 45.4±2 ms; P<0.05) but significantly reduced by both treatments (Omacor and aliskiren: 49±1.5 and 49.3±1.6 ms, P<0.05, respectively; Figure 3B and 3C).

Disturbance in the ventricular refractory period indicated changes in the electrophysiological phenotype, which was confirmed by ECG and MCG. The prolonged QRS interval in dTGRs compared with SD controls (21±0.5 versus 18.4±0.2 ms; P<0.05; Figure 4A) was similarly reduced by both treatments (Omacor and aliskiren: 19.1±0.1 and 19±0.3 ms, P<0.05, respectively). In contrast, the acquired long QT syndrome in dTGRs (QTpeak and QTc: 42±1 and 109±3 ms versus SD: 30±1 and 75±3 ms, P<0.05, respectively) was only prevented by aliskiren treatment (31±1 and 79±3 ms; Figure 4B and 4C). However, Omacor reduced the repolarization period (QTpeak and QTc: 37±1 and 99±2 ms, P<0.05, respectively). T-wave dispersion assessed by MCG surface mapping was slightly reduced in Omacor-treated compared with untreated dTGRs (12.9±0.5 versus 14.5±0.6 ms; P<0.05; Figure 4D). Aliskiren completely normalized QTpeak dispersion (6±0.7 versus SD 5.9±0.8 ms).

Untreated dTGRs showed marked fibrosis. Cardiac immunoreactivity for collagen I was observed predominantly perivascularly, whereas fibronectin expression was predominantly interstitial. Aliskiren treatment and n-3 PUFA supplementation substantially reduced both matrix depositions (Figure 5A and 5B). ED-1, a marker of monocyte/macrophage infiltration, showed prevalent inflammation in untreated dTGRs. Both treatments reduced monocyte/macrophage infiltration to the SD level (Figure 5C). Cx43 gap junction dislocation is a mechanism of dTGR electrical remodeling. We found pronounced Cx43 immunoreactivity at the lateral cardiomycocyte borders (Figure 5D). In contrast, Cx43 was restricted to the intercalated disc regions in SD. Surprisingly, hypertrophied hearts of Omacor-treated dTGRs showed normal Cx43 gap junction localization. Aliskiren also prevented abnormal Cx43 expression.

**Figure 2.** A, Untreated dTGRs showed high mortality (5 of 16), which was reduced to 0 by Omacor (0 of 18) and aliskiren (0 of 9) treatment. None of the SD controls died (n=10). B, At the age of 7 weeks, Omacor reduced blood pressure only slightly, whereas aliskiren normalized it. C, Untreated and Omacor-treated dTGRs both showed increased cardiac weight compared with aliskiren-treated dTGRs and SD rats. D, Left-ventricular ANP mRNA expression, as a marker for cardiac hypertrophy, confirmed the results. Results are mean±SEM. *P<0.05 vs untreated dTGRs; # P<0.05 vs Omacor rats.
Discussion

We showed that an n-3 PUFA–based intervention reduced mortality in our model to 0. n-3 PUFA supplementation reduced electrophysiological alterations to a similar extent as the DRI aliskiren, despite a 70-mm Hg difference in blood pressure and the development of cardiac hypertrophy. These results indicate that the levels of blood pressure and cardiac hypertrophy are not the sole determinants for increased arrhythmias. Both treatments prevented inflammation, fibrosis, and dyslocalization of Cx43 gap junctions. Therefore, reduction in arrhythmias or sudden death may depend on a nonmorphological tissue architecture and organized cell-cell coupling compared with cardiac hypertrophy, per se.

DRI now offers a novel opportunity in treating hypertensive heart disease. Aliskiren was recently approved for the treatment of hypertension.20,21 Aliskiren strongly reduced ventricular tachycardia induction, QRS, and QT prolongation, and increased T-wave dispersion. To our knowledge, this

Figure 3. In vivo electrophysiological studies in 7-week–old dTGRs (n=8), dTGR+Omacor (n=9), dTGR+aliskiren (n=8), and SD (n=7) rats. A, The figure shows inducibility of ventricular arrhythmias by in vivo–programmed electrical stimulation. Given is the number of stimulation protocols with inducible arrhythmias from all of the performed stimulation protocols in percentages. B, The left-ventricular effective refractory period was prolonged in untreated dTGRs. Both treatments reduced ventricular refractory. *P<0.05 vs dTGRs; # P<0.05 vs dTGR+Omacor rats, . . . . P<0.05 vs dTGR+aliskiren) C, Given are representative results of programmed electrical stimulation from all of the groups. One ECG is shown simultaneously with indicators of delivered electrical stimuli (Stim). Untreated dTGRs showed reproducible inducibility of ventricular tachyarrhythmias, whereas in most cases the same stimulations showed no inducibility in both treatment groups. In SD controls, no arrhythmias were inducible.

Figure 4. A, QRS interval was significantly prolonged in untreated dTGRs. Both treatments reduced QRS intervals to the same extent. B and C, Repolarization was prolonged in untreated dTGRs indicated by an acquired long QT syndrome. Aliskiren normalized QT intervals, whereas Omacor reduced QT intervals. D, The temporal dispersion of the peak of T wave was assessed by magnetocardiographic surface mapping. The figure gives the maximal difference in Tpeak within a square area of 60x60 mm over the animal's thorax. The high TPeak dispersion of untreated dTGRs was significantly reduced by Omacor treatment. Aliskiren reduced it to control levels. *P<0.05 vs dTGRs, # P<0.05 vs dTGR+Omacor rats. These data were obtained using the same animals as in Figure 3.
report is the first study demonstrating the impact of DRI in Ang II–induced electrical remodeling. Renin-angiotensin system inhibition is 1 of the important strategies to prevent electrical remodeling–based arrhythmias. Ang II promotes the release of aldosterone. Both hormones are strong inducers of fibrosis and inflammation that contribute to the pathogenesis of heart failure and electrical remodeling. Furthermore, Ang II and aldosterone affect Ca\textsuperscript{2+} cycling, decrease conduction velocity as a result of cell-to-cell-uncoupling, and lead to increased dispersion of repolarization by inhibiting potassium channels.\textsuperscript{22} In humans, angiotensin-converting enzyme inhibitors, angiotensin type 1 receptor blockers, and mineralocorticoid receptor blockers all exhibited antiarrhythmic potential.\textsuperscript{23} Currently, no human study has addressed the effect of DRI on electrical remodeling. We did not measure plasma renin activity and aldosterone levels in this study, but showed earlier that aliskiren reduces plasma renin activity.\textsuperscript{16} We do not believe that Omacor reduced plasma renin activity, because its blood pressure effect was modest.

In humans, n-3 PUFAs decreased the risk of sudden cardiac death from ventricular arrhythmias in coronary artery–diseased patients.\textsuperscript{11,24–26} The Gruppo Italiano per lo Studio della Sopravvivenza nell’Infarto-Prevenzione Study showed a 45% reduction in sudden cardiac death in patients with recent myocardial infarction.\textsuperscript{25} In patients after coronary artery bypass graft surgery, n-3 PUFAs reduced atrial fibrillation by almost 55%.\textsuperscript{26} This antiarrhythmic effect also applies to our model of Ang II–induced sudden cardiac death. n-3 PUFAs was as effective in reducing mortality and arrhythmia induction as was DRI. This finding is surprising, because n-3 PUFA–treated dTGRs had 70-mm Hg higher blood pressure, as well as unchanged cardiac hypertrophy, with elevated ANP expression. The slight reduction in blood pressure by n-3 PUFA in dTGRs is in agreement with the antihypertensive effect described in other studies. We believe that blood pressure and cardiac hypertrophy are not the sole determinants for our electrophysiological phenotype. First, both treatment strategies reduced the likelihood of arrhythmias despite a 70-mm Hg blood pressure difference. We showed in a previous study in the same model that triple therapy with hydralazine, reserpine, and hydrochlorothiazide normalized blood pressure but just delayed and did not prevent inflammatory cardiac damage.\textsuperscript{16} Second, studies in mice with restricted cardiac high Ang II levels and heart-specific mineralocorticoid receptor overexpression showed sudden cardiac death, arrhythmias, and electrical remodeling despite normal blood pressure.\textsuperscript{27–29} Taken together these results support the hypothesis that Ang II increases the risk for arrhythmias by blood pressure–dependent and independent mechanisms, which can be inhibited by aliskiren and n-3 PUFA treatment.

The marked reductions in the QRS interval and in arrhythmia induction suggest an improvement in conduction. The factors most likely responsible are fibrosis and Cx43 gap junctions, which are crucial for electrical coupling of adjacent cardiomyocytes. Indeed, we found reduced fibrosis and, more surprisingly, a normal subcellular distribution of Cx43 in both treatment groups. Ang II–induced gap junction dyslocalization in untreated dTGRs was probably due to changes in the Cx43 phosphorylation state.\textsuperscript{30} Cx43 redistribution also occurs under conditions of ischemia and contributes to electrical uncoupling of cardiomyocytes and the development of arrhythmias. Cardiac preconditioning was shown to prevent intracellular Cx43 redistribution and to protect against cardiac injury and arrhythmias during subsequent attacks of prolonged ischemia.\textsuperscript{31} We are the first to show that there is also a direct link between the antiarrhythmic properties of n-3 PUFAs and restoration of proper Cx43 localization to the
intercalated disks. DHA also improved the functional localization of Cx43 in astrocytes, indicating the existence of common mechanisms of how n-3 PUFAs may improve gap junction coupling in heart and brain cells.

The modulation of cellular electrophysiology by n-3 PUFAs further includes the reduction of membrane excitability by modulating the function of specific ion channels and the Ca²⁺ release from the sarcoplasmic reticulum. A potential source of biologically active metabolites produced in AA-derived eicosanoids. Among these metabolites, cytochrome P450–dependent epoxy eicosatrienoic acids but is largely exceeded by their EPA- and DHA-derived counterparts. Enhanced epoxyeicosatrienoic acid generation was shown to exert cardioprotective effects via the principal capacity of KATP channel activation and are essential for the central role in cardiac protection and the reduction of membrane excitability by modulating the function of specific ion channels and the Ca²⁺ release from the sarcoplasmic reticulum. A similar amelioration of repolarization parameters was demonstrated in our study. However, the distinct molecular pathways by which n-3 PUFAs exert their antarrhythmic effects and reduce the risk of sudden cardiac death are unknown. n-3 PUFAs may directly interact with certain cellular targets or change the microenvironment of membrane-bound signaling components after being incorporated into phospholipids. Moreover, n-3 PUFAs are substrates for cytochrome P450, cyclooxygenase, and lipoygenase enzymes. Thus, depending on the diet, n-3 PUFAs are a potential source of biologically active metabolites produced in competition with AA-derived eicosanoids. Among these metabolites, cytochrome P450–dependent epoxy eicosatrienoic acids but is largely exceeded by their EPA- and DHA-derived counterparts. Enhanced epoxyeicosatrienoic acid generation was shown to exert cardioprotective effects via the principal capacity of KATP channel activation. The effects of n-3 PUFAs are in part mediated by cytochrome P450–dependent formation of alternative highly potent KATP channel activators.

**Perspectives**

DRI and n-3 PUFAs may be future potent therapeutic agents providing cardioprotection and reduction in the risk of arrhythmias in particular. The combination of both therapies might be useful in patients with hypertension-induced heart disease. The discovery of the n-3 PUFA signaling pathway may present new candidates for antiarrhythmic drugs.

**Acknowledgments**

We thank Astrid Schiche, Jutta Meisel, and Gabriele N’diaye for technical assistance.

**Sources of Funding**

Grants-in-Aid from the European Union (EuReGene), Solvay Pharmaceuticals, the Novartis Foundation, and the Deutsche Forschungsgemeinschaft to W.H.S., D.N.M. and F.C.L. supported the studies.

**Disclosures**

F.C.L. and D.N.M. have served as advisors for Novartis and have lectured on aliskiren. F.C.L. is a member of the Renin Academy. The remaining authors report no conflicts.

**References**


Dietary n-3 Polyunsaturated Fatty Acids and Direct Renin Inhibition Improve Electrical Remodeling in a Model of High Human Renin Hypertension


Hypertension. 2008;51:540-546; originally published online December 24, 2007; doi: 10.1161/HYPERTENSIONAHA.107.103143

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2007 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/51/2/540

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/