European Review for Medical and Pharmacological Sciences 2013; 17: 2632-2638

Regulatory functions of docosahexaenoic acid on ion channels in rat ventricular myocytes

Y.-J. SONG, P.-S. DONG, H.-L. WANG, J.-H. ZHU, S.-Y. XING, Y.-H. HAN, R.-X. WANG[1], W.-P. JIANG[2]

Department of Cardiology, First Affiliated Hospital of Henan University of Science and Technology, Luoyang, China.
[1]Department of Cardiology, Wuxi People's Hospital Affiliated to Nanjing Medical University, Wuxi, China.
[2]Department of Cardiology, First Affiliated Hospital of Soochow University, Suzhou, China.

Abstract. – OBJECTIVE: The aim of this study was to study the regulatory functions of docosahexaenoic acid (DHA) on resting potential (RP), action potential duration (APD), delayed rectifier potassium current (I_k), and inwardly rectifier potassium current (I_{k1}) in rat ventricular myocytes, and analyze the related anti-arrhythmia mechanism.

MATERIALS AND METHODS: Rat ventricular myocytes were isolated by enzyme digestion method. RP, APD, I_k and I_{k1} in individual ventricular myocytes were recorded by patch-clamp technique with whole-cell configuration. Effects of DHA with various concentrations (0, 20, 40, 60, 80, 100 and 120 μmol/L, respectively) on RP, AP, I_k and I_{k1} were investigated.

RESULTS: There was no statistical difference of RP with different DHA concentrations ($p > 0.05$, $n = 20$), and the 25%, 50% and 90% of APD (APD_{25}, APD_{50}, and APD_{90}) were gradually prolonged with increase of DHA concentration, respectively ($p < 0.05$, $n = 20$). I_K gradually blocked and the I-V curve was downward shifted, according to increase of DHA concentration ($p < 0.05$, $n = 20$). The DHA half effect concentration (EC_{50}) was 47.52 ± 2.32 μmol/L. With increasing DHA concentration, the steady-state inactivation curve shifted to left, and the recovery curve shifted to right. DHA had no significant effect on I_{K1} ($p > 0.05$, $n = 20$).

CONCLUSIONS: DHA has regulatory functions on RP, APD, I_k and I_{k1} in rat ventricular myocytes, which may be one of the related antiarrhythmic mechanisms.

Key Words:

Docosahexaenoic acid, Arrhythmia, Regulatory function.

Introduction

Fatty acids, especially n-3 polyunsaturated fatty acids (n-3PUFAs) are not only one of important energy sources of heart, but also play an important role in electrophysiological activities and metabolic processes of myocardial cells. Several epidemiological and clinical studies find that, daily intake of a certain amount of n-3 PUFAs can obtain the hypolipidemic and antithrombotic effects, improve the immune function[1,2], and prevent the cardiovascular diseases[3-6], especially for malignant arrhythmia. The effects of n-3 PUFAs on prevention of cardiovascular diseases and sudden cardiac death have caused more attention[6,7-10]. Animal experimental results have also showed the antiarrhythmic effect of n-3PUFAs[11]. Based on these research results, daily intake of low-dose n-3 PUFAs (about 1 g) is recommended by Society of Cardiology in USA and European countries for primary and secondary prevention of cardiovascular diseases[12,13].

Myocardial cell action potential (AP) is usually divided into 5 phases as follows: phase 0 (rapid depolarization phase), mainly formed by rapid inward sodium current; phase 1 (rapid repolarization phase), mainly formed by transient outward potassium current (I_{to}); phase 2 (platform phase), mainly formed by inward calcium current ($I_{Ca\text{-}L}$) and delayed rectifier potassium current (I_K); phase 3 (rapid repolarization phase), mainly formed by I_K; phase 4 (resting phase), mainly formed by inward rectifier potassium current (I_{K1}). The action potential duration (APD) of myocardial cells depends on interaction of inward current and outward current. The increased inward current and/or decreased outward current can cause the extension of APD. APD_{25} (early repolarization) is mainly decided by I_{to}, and APD_{50} is mainly decided by $I_{Ca\text{-}L}$ and I_K. APD_{90} is mainly influenced by I_K, and partially by I_{K1}.

At present, the antiarrhythmic mechanism of n-3 PUFAs is still not entirely clear. n-3 PUFAs mainly include docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Clinical experiments find that, DHA is easier to accumulate in

Corresponding Author: Ping-Shuan Dong, MD; e-mail: pingshuandong@yeah.net

cardiac tissue, and has important effects such as coronary artery dilatation, improving blood circulation, reducing blood pressure, antiarrhythmia and prevention of cardiovascular diseases[14]. In this study, the regulatory functions of DHA on resting potential (RP), APD, I_k, and (I_{k1}) in rat ventricular myocytes were investigated, and the related antiarrhythmic mechanism was analyzed.

Materials and Methods

DHA effects on RP and APD

The single ventricular myocytes of Sprague Dawley (SD) rat was isolated by enzyme digestion method[15]. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of First Affiliated Hospital of Henan University of Science and Technology. The cell suspension was put in cellular pool. A positive voltage was imposed on the electrode before entering cell suspension to compensate the liquid junction potential. The high-resistance sealing between electrode and cell membrane was formed. The electrode capacitance was compensated and the cell membrane was ruptured under negative voltage. The series resistance and cell membrane capacitance were compensated. In the current-clamp mode of "I = 0", RP was directly shown on the display screen of amplifier. The APD was recorded in the current-clamp mode as follows: impulse width, 5 m; stimulus current, 900 pA; stimulus frequency, 1 Hz. The effects of DHA (0, 20, 40, 60, 80, 100 and 120 μmol/L, respectively) on RP and APD were observed. 2 mg/mL bovine serum albumin (BSA) was used to elute DHA. The experiment was conducted at room temperature (20-22 °C)[16].

DHA effects on IK

Under the voltage-clamp mode, the stimulus voltage was gradually depolarized from holding potential (HP) (-50 mV) to +60 mV (phase step, +10 mV; duration, 7 s; stimulus frequency, 0.1 Hz). The I_K was recorded, and the I_K peak was obtained. The I-V curve (current density against potential) was created. The effects of DHA with different concentrations on I_K were observed. 2 mg/mL BSA was used to elute DHA.

DHA effects on steady-state activation curve of delayed rectifier potassium channel (DRPC)

The HP was kept as 50 mV, and the stimulus potential was gradually depolarized from -30 mV to +60mV (phase step, +10 mV; duration, 7 s; stimulus frequency, 0.1 Hz). The activated I_K was recorded. The steady-state activation curve of current relative value (I/I_{max}) against stimulus potential was created. The half-activation voltage and slope were obtained according to Boltzmann equation. The effects of DHA with different concentration on I_K activation were observed.

DHA effects on inactivated current and steady-state inactivation curve of DRPC

The HP was kept as 70 mV. The pulse stimulation from -100 mV to 0 mV (phase step, +10 mV; duration, 1000 ms) was imposed. Each pulse was followed by a fixed test pulse (depolarized to +30 mV, 150 ms), then the potential restored to -70 mV. The inactivated current of I_K was recorded, and the steady-state inactivation curve of current relative value (I/I_{max}) against stimulus potential was created. The half-inactivation voltage and slope were obtained according to Boltzmann equation. The effects of DHA with different concentration on I_K inactivation were observed.

DHA effects on recovered current and recovery curve after inactivation of DRPC

The HP was kept as -50 mV. The +50 mV pulse stimulation was imposed for 2500 ms, followed by repolarization to -50 mV. The second +50 mV stimulation was conducted with interval time of 50, 100, 200, 400, 800, and 1600 ms, respectively. The recovered current after I_K inactivation was recorded. The recovery current with the second pulse stimulation was compared to conditioned stimulus current, and the recovery curve of current relative value (I/I_{max}) against interval time was created. The recovered time constant τ was obtained according to single exponential equation. The effects of DHA with different concentration on recovery after I_K inactivation were observed.

DHA effects on I_{K1}

The HP was kept as - 60 mV. The pulse stimulation from -100 mV to 0 mV (phase step, +10 mV; duration, 150 ms; stimulation frequency, 0.2 Hz) was imposed. The I_{K1} was recorded. The effects of DHA with different concentration on I_{K1} were observed. 2 mg/mL BSA was used to elute DHA.

Table I. Effects of DHA with different concentrations on RP and APD (n = 10).

Index	DHA concentration (mmol/L)						
	0	20	40	60	80	100	120
RP (mV)	-76.0±5.3	-74.4±4.7[a]	-75.7±4.8[a]	-76.9±6.1[a]	-76.6±5.6[a]	-74.6±4.9[a]	-78.6±7.7[a]
APD_{25} (ms)	4.9±1.4	5.8±1.9	7.3±2.2	11.0±3.3[b]	13.3±4.2[b]	14.4±5.0[b]	15.8±5.3[b]
APD_{50} (ms)	11.8±2.2	13.2±3.0	14.6±3.4	18.9±4.2[c]	20.5±4.8[c]	22.2±5.3[c]	23.9±5.7[c]
APD_{90} (ms)	52.8±5.3	56.6±5.7	59.2±6.1	88.5±8.9[d]	103.4±10.3[d]	121.6±11.8[d]	133.7±12.3[d]

Note: compared to 0 μmol/L DHA, [a]$p > 0.05$, [b]$p < 0.05$, [c]$p < 0.05$, [d]$p < 0.05$

Statistical analysis

Data were expressed as mean ± SD. Statistical analysis was performed using SPSS 11.5 statistical software. A *t*-test was used to analyze the differences between two groups, and $p < 0.05$ was considered as statistically significant. The half-activation voltage, half-inactivation voltage, slope and half effect concentration (EC50) were obtained according to Boltzmann equation using Originpro 7.5 graphing and data analysis software.

Results

Effects of DHA on RP and APD

As shown in Table I, there was no statistical difference of RP among different DHA concentrations (0, 20, 40, 60, 80, 100 and 120 μmol/L, respectively) ($p > 0.05$, $n = 20$). The 25%, 50% and 90% of APD (APD_{25}, APD_{50}, and APD_{90}) were gradually prolonged with increase of DHA concentration, respectively ($p < 0.05$, $n = 20$).

Effects of DHA on I_K

Under action of DHA, there was a concentration-dependent inhibition of I_K and peak current, respectively ($p < 0.05$, $n = 20$) (Figure 1A), with downward shift of I-V curve according to increase of DHA concentration (Figure 1B). The inhibition rate of I_K by DHA with different concentrations were 0, (2.78 ± 0.26) %, (27.23 ± 3.97) %, (64.18 ± 6.73%), (77.59 ± 7.36) %, (83.26 ± 8.31) % and (87.93 ± 9.35%), respectively ($p < 0.05$, $n = 20$). The EC_{50} of DHA to I_K according to Boltzmann equation was (47.52 ± 2.32) μmol/L (Figure 1C).

Effects of DHA on steady-state activation curve of DRPC

Figure 2 showed that, the steady-state activation curve of I_K did not significantly change under action of DHA with different concentrations, with no statistical difference of half-activation voltage. The half-activation voltage and slope with different concentration of DHA were as follows: (35.71 ± 3.81) mV, 12.01 ± 0.57; (37.35 ± 4.46) mV, 12.22 ± 0.81; (39.98 ± 5.09) mV, 13.87 ± 1.03; (39.79 ± 4.95) mV, 14.13 ± 1.06; (40.86 ± 5.36) mV, 17.51 ± 1.55; (39.95 ± 5.02) mV, 16.56 ± 1.39; (37.32 ± 4.38) mV, 12.33 ± 0.85 ($p > 0.05$, $n = 20$).

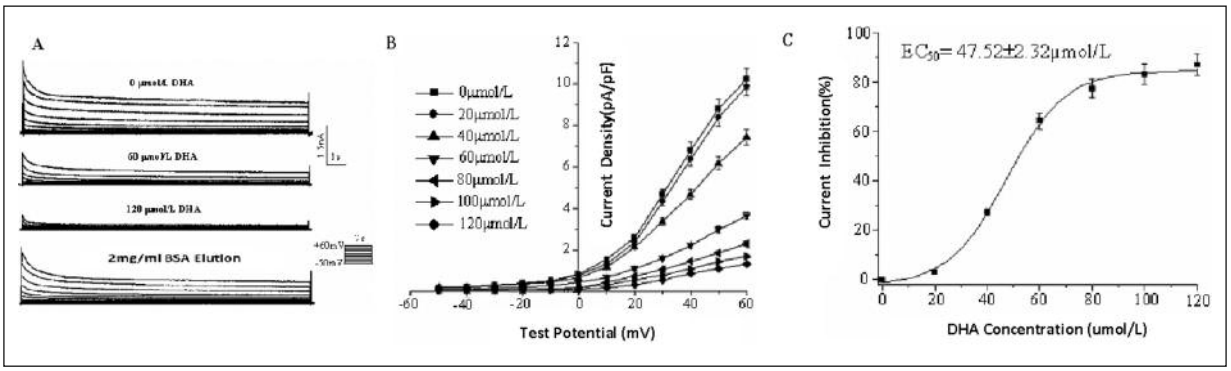

Figure 1. A, Decrease of I_k at the effect of 0, 60 and 120 μmol/L DHA, respectively. Recover of I_k through 2 mg/mL BSA used to elute DHA. **B**, *I-V* curves of I_k at different DHA concentrations and test potentials. **C**, Percentages of I_k decreased at different DHA concentrations.

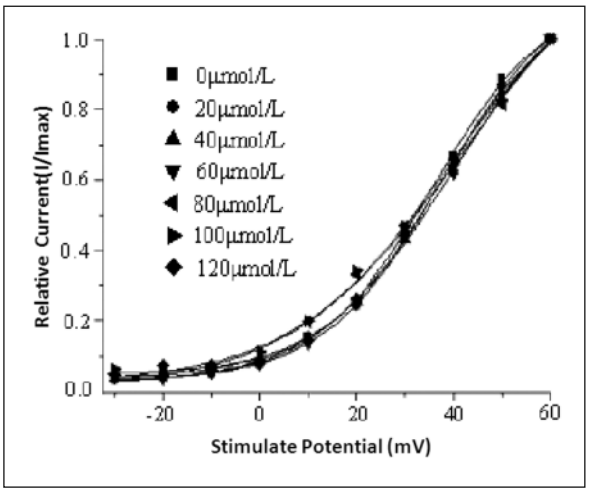

Figure 2. Stably activated curves of I_k at different DHA concentrations.

Effects of DHA on inactivated current and steady-state inactivation curve of DRPC

Under action of DHA, the inactivated current amplitude of I_K decreased (Figure 3A). The steady-state inactivation curve shifted to left (hyperpolarization) according to increase of DHA concentration, and the absolute value of inactivation voltage increased (Figure 3B). The half-inactivation voltage and slope with different concentration of DHA were as follows: (-33.36 ± 1.07) mV, 11.75 ± 0.64; (-34.45 ± 1.36) mV, 11.49 ± 0.61; (-37.19 ± 2.03) mV, 11.27 ± 0.59; (-40.02 ± 2.87) mV, 10.56 ± 0.54; (-41.31 ± 3.32) mV, 10.15 ± 0.51; (-42.82 ± 3.95) mV, 10.79 0.55; (-43.57 ± 5.52) mV, 9.83 ± 0.49 ($p < 0.05$, $n = 20$).

Effects of DHA on recovered current and recovery curve after inactivation of DRPC

Under action of DHA, the recovered current amplitude after I_K inactivation decreased (Figure 4A). The recovery curve shifted to right, and τ increased with the increase of DHA concentration (Figure 4B). The values of with different concentration of DHA were as follows: (168.18 ± 16.67) ms, (175.59 ± 17.73) ms, (207.29 ± 20.13) ms, (259.96 ± 28.47) ms, (286.30 ± 31.19) ms, (305.53 ± 33.97) ms, and (316.41 ± 35.24) ms ($p < 0.05$, $n = 20$).

Effects of DHA on I_{K1}

As shown in Figure 5, there was no significant change of I_{K1} current amplitude under action of DHA. The current density with different concentration was (-31.6 ± 6.8) pA/pF, (-36.3 ± 8.7) pA/pF, (-30.9 ± 6.2) pA/pF, (-34.2 ± 7.1) pA/pF, (-29.8 ± 5.7) pA/pF, (-28.6 ± 5.1) pA/pF and (-33.4 ± 6.9) pA/pF, respectively, with no statistical difference among them ($p > 0.05$, $n = 20$).

Discussion

Clinical studies show that, daily intake of certain amount of n-3PUFAs has benefit of reducing blood lipid, anti-thrombosis, and stabilizing atherosclerotic plaque[2,17]. In addition, it can significantly reduce the incidence of sudden death in secondary prevention of coronary heart disease, and reduces the mortality after myocardial infarction and occurrence of malignant arrhythmia[18,19]. Leaf et al[20] have studied 420 patients implanted with ICD (implanted cardioverter defibrillator) and find that, n-3PUFAs can significantly reduce the appearance of fatal ventricular arrhythmia. Xiao et al[21] showed that, after application of DHA in porcine models with acute myocardial infarction, the incidence of malignant arrhythmia and myocardial infarction area were significantly reduced. Mozaffarian et al[22] have

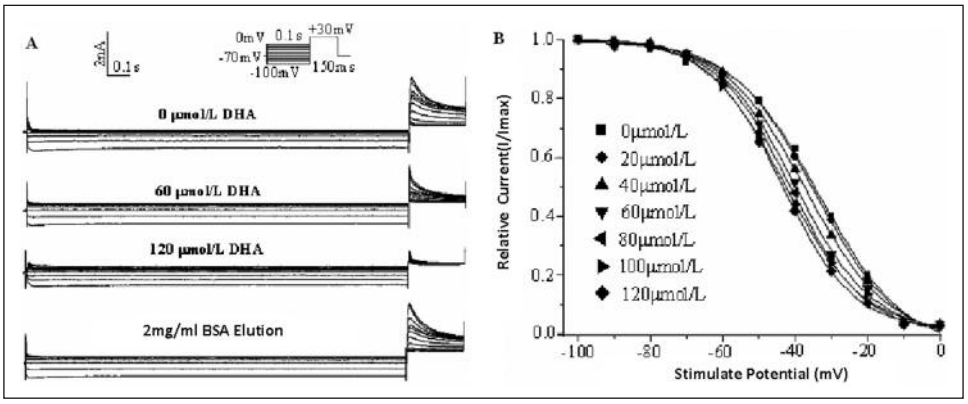

Figure 3. A, Stably inactivated I_k at the effect of 0, 60 and 120 μmol/L DHA, respectively. Recover of stably inactivated I_k through 2 mg/mL BSA used to elute DHA. **B**, Stably inactivated curves of I_k at different DHA concentrations.

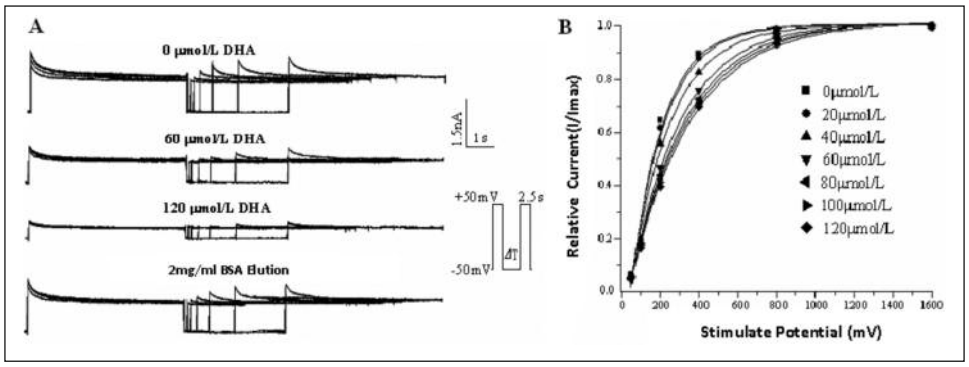

Figure 4. A, Recovered I_k from inactivation at the effect of 0, 60 and 120 μmol/L DHA, and 2 mg/mL BSA used to elute DHA. **B**, Recovered I_k curves from inactivation at different DHA concentrations.

studied 4815 patients (≥ 65 years old) with atrial fibrillation history and find that, after intake of 2.2 g of n-3PUFAs (1-4 times per week) for 1 month, the prevalence of atrial fibrillation decreased by 28% (decreased by 31%, with intake 5 times per week). This suggests that, n-3PUFAs can reduce the occurrence of atrial fibrillation, with a dose-dependence. At present, the antiarrhythmic mechanism of n-3 PUFAs is not completely clear. N-3 PUFAs may affect the ion channel, inhibit the triggered activity, reduce the myocardial ischemic injury, activate the protein kinase A, and improve the postischemic contractile function[23-28]. Leaf et al[29] believe that, the antiarrhythmic function of n-3PUFAs is closely related to the effect on sodium channel of ventricular myocytes and prolonging effect on relative refractory period of AP.

Potassium channel is the primary ion current of AP repolarization in ventricular myocytes. The change of potassium channel can significantly affect the AP shape and APD. This channel is also the main target site for many drugs. There are many types and subtypes of potassium channel in ventricular myocytes, with more than 10 types of voltage-dependent potassium channel, which are divided into K_V channel and K_{IR} (IRK) channel[30]. K_V channel is composed of 4 independent transmembrane segments. Each transmembrane segment (α subunit) consists of 6 α helices (S1-S6). The H5 ring (S5-S6 connection) is the site for formation of potassium channel pore and binding of drug and toxin. The β subunit plays an important role in inactivation of potassium channel. KV channel is also divided into 4 subtypes, namely Kv1, Kv2, Kv3, and Kv4. The Kv1.4, Kv4.2, and Kv4.3 clones are related to transient outward potassium current (I_{to}). The minK and KvLQT1 clones together decide the formation of I_K, while the formation of I_{K1} is decided mainly by hIRK clone. Each α subunit of IRK channel is composed of only 2 transmembrane segments (M1, M2) connected by H5. As the sequence of M1, M2 and H5 is similar with S5, S6 and H5 in Kv, respectively, the basic pore structures in these two channels are the same. However, due to absence of S4 like structure, the gating mechanism of IRK is different with KV, though the voltage dependence exists. With the voltage dependence and receptor dependent, IRK channel has inward rectifier characteristics.

In this study, DHA can inhibit I_K, promote channel inactivation, and extend recovery time from inactivation state, with no effect on channel activation. I_K has important significance on termination of AP plateau phase and the phase 3 repolarization. The inhibition of I_K by DHA causes the extension of APD (especially APD_{50} and APD_{90}), repolarization time, and effective refractory period, leading to termination of various microreentries. This may be one of antiarrhythmic mechanisms of DHA. This study also shows that, APD_{25}, APD_{50} and APD_{90} are gradually prolonged with the increase of DHA concentration. This indicates that, DHA also has effect on other ion

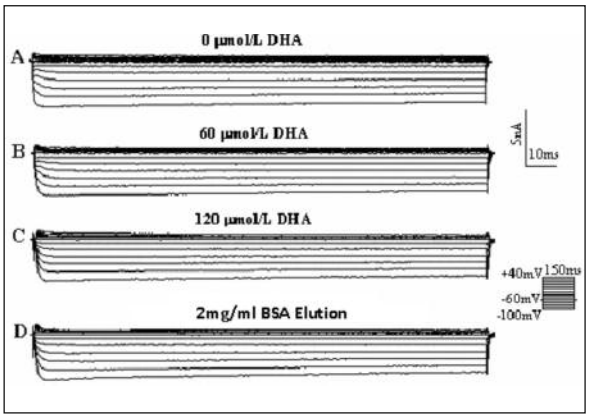

Figure 5. I_{k1} effected by 0, 60 and 120 μmol/L DHA, and 2 mg/mL BSA used to elute DHA.

channels for AP formation. As found in our previous studies, DHA can affect the sodium channel and transient outward potassium channel[15,31].

I_{K1} is the main ion current deciding RP in ventricular myocytes, and plays an important role in rapid repolarization of AP[32]. The I_{K1} density is the highest in ventricular muscle and Purkinje fibers, and lower in atrium and sinoatrial node cells[33]. I_{K1} has inward rectifier property. The outward potassium current amplitude decreases with the increase of depolarized membrane potential. The physiological functions of I_{K1} are related to the inward rectifier property. When the cell membrane is in state of mild depolarization, the I_{K1} channel is opened, and a certain amount of outward current is produced to maintain the RP[34]. Further depolarization causes I_{K1} channel closure and current amplitude decrease. So other ion currents in last stage of phase 3 repolarization of AP are inactivated, and the channels are activated by repolarized potential. The outward potassium current increases, and become the main component of the outward current. The cells are rapidly repolarized to RP level, and the early depolarization is avoided. The influence on I_{K1} can change the RP, and increase the incidence of arrhythmias[35]. As found in this study, DHA has no significant effect on I_{K1}, so the RP is not influenced. This study also shows that, DHA can not affect the RP. DHA can inhibit the delayed rectifier potassium channel, with no effect on inward rectifier potassium channel. This may be related to structure difference of two kinds of potassium channel. The actions of DHA on APD, delayed rectifier potassium channel and inward rectifier potassium channel may be one of the related antiarrhythmia mechanisms.

Conclusions

At present, whether DHA directly acts with α or β subunit in potassium channel is still not clear. Boland et al[36] believe that, n-3PUFAs can bind with the phospholipid bilayer in cell membrane, and cause change of cell membrane fluidity, thus affecting the channel in ventricular myocytes. Pound et al[37] believe that, the concentration of n-3PUFAs binding cell membrane is very low. Low-concentration n-3PUFAs can not regulate the ion channel. However, n-3PUFAs may assemble in a certain region on cell membrane, and affect the transmembrane parts of ion channel. The action pathway of DHA on ion channel still needs to be further investigated.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Calder PC. n-3 Fatty acids and cardiovascular disease: evidence explained and mechanisms explored. Clin Sci (Lond) 2004; 107: 1-11.
2) Wang C, Harris WS, Chung M, Lichtenstein AH, Balk EM, Kupelnick B, Jordan HS, Lau J. n-3 Fatty acids from fish or fish-oil supplements, but not alpha-linolenic acid, benefit cardiovascular disease outcomes in primary – and secondary – prevention studies: a systematic review. Am J Clin Nutr 2006; 84: 5-17.
3) Wilhelm M, Tobias R, Asskali F, Kraehner R, Kuly S, Klinghammer L, Boehles H, Daniel WG. Red blood cell omega-3 fatty acids and the risk of ventricular arrhythmias in patients with heart failure. Amer Heart J 2008; 155: 971-977.
4) Arshad A, Mandava A, Kamath G, Musat D. Sudden cardiac death and the role of medical therapy. Prog Cardiovasc Dis 2008; 50: 420-438.
5) Anand RG, Alkadri M, Lavie CJ, Milani RV. The role of fish oil in arrhythmia prevention. J Cardiopulm Rehabil Prev 2008; 28: 92-98.
6) Metcalf RG, Sanders P, James MJ, Cleland LG, Young GD. Effect of dietary n-3 polyunsaturated fatty acids on the inducibility of ventricular tachycardia in patients with ischemic cardiomyopathy. Am J Cardiol 2008: 101: 758-761.
7) Madsen T, Christensen JH, Thøgersen AM, Schmidt EB, Toft E. Intravenous infusion of n-3 polyunsaturated fatty acids and inducibility of ventricular tachycardia in patients with implantable cardioverter defibrillator. Europace 2010; 12: 941-946.
8) Xiao YF, Ma L, Wang SY, Josephson ME, Wang GK, Morgan JP, Leaf A. Potent block of inactivation-deficient Na+ channels by n-3 polyunsaturated fatty acids. Am J Physiol Cell Physiol 2006; 290: C362-370.
9) Harris WS, Kris-Etherton PM, Harris KA. Intakes of long-chain omega-3 fatty acid associated with reduced risk for death from coronary heart disease in healthy adults. Curr Atheroscler Rep 2008; 10: 503-509.
10) Patel JV, Tracey I, Hughes EA, Lip GY. Omega-3 polyunsaturated fatty acids: a necessity for a comprehensive secondary prevention strategy. Vasc Health Risk Manag 2009; 5: 801-810.
11) Balk EM, Lichtenstein AH, Chung M, Kupelnick B, Chew P, Lau J. Effects of omega-3 fatty acids on serum markers of cardiovascular disease risk: a systematic review. Atherosclerosis 2006; 189: 19-30.
12) von Schacky C, Harris WS. Cardiovascular benefits of omega-3 fatty acids. Cardiovasc Res 2007; 73: 310-315.

13) Harris WS. International recommendations for consumption of long-chain omega-3 fatty acids. J Cardiovasc Med (Hagerstown) 2007; 8: S50-52.

14) Xiao YF, Sigg DC, Leaf A. The antiarrhythmic effect of n-3 polyunsaturated fatty acids: modulation of cardiac ion channels as a potential mechanism. J Membr Biol 2005; 206: 141-154.

15) Wang RX, Li XR, Lai LH, Wu XQ, Chen YF, Song JP, Yang XJ, Jiang WP. Effects of docosahexaenoic acid on action potential and transient outward potassium current on ventricular myocytes of Sprague-Dawley rat. Zhonghua Xin Xue Guan Bing Za Zhi 2009; 37: 108-111.

16) Stadnicka A, Marinovic J, Bienengraeber M, Bosnjak ZJ. Impact of in vivo preconditioning by isoflurane on adenosine triphosphate-sensitive potassium channels in the rat heart: lasting modulation of nucleotide sensitivity during early memory period. Anesthesiology 2006; 104: 503-510.

17) Calder PC, Yaqoob P. Omega-3 (n-3) fatty acids, cardiovascular disease and stability of atherosclerotic plaques. Cell Mol Biol 2010; 56: 28-37.

18) Marchioli R, Silletta MG, Levantesi G, Pioggiarella R, Tognoni G. N-3 polyunsaturated fatty acids in heart failure: mechanisms and recent clinical evidence. Cell Mol Biol 2010; 56: 110-130.

19) Deckelbaum RJ, Leaf A, Mozaffarian D, Jacobson TA, Harris WS, Akabas SR. Conclusions and recommendations from the symposium, Beyond Cholesterol: Prevention and Treatment of Coronary Heart Disease with n-3 Fatty Acids. Am J Clin Nutr 2008; 87: 2010S-2012S.

20) Leaf A, Albert CM, Josephson M, Steinhaus D, Kluger J, Kang JX, Cox B, Zhang H, Schoenfeld D. Prevention of fatal arrhythmias in high-risk subjects by fish oil n-3 fatty acid intake. Circulation 2005; 112: 2762-2768.

21) Xiao YF, Sigg DC, Ujhelyi MR, Wilhelm JJ, Richardson ES, Iaizzo PA. Pericardial delivery of omega-3 fatty acid: a novel approach to reducing myocardial infarct sizes and arrhythmias. Am J Physiol Heart Circ Physiol 2008; 294: H2212-2218.

22) Mozaffarian D, Psaty B, Rimm EB, Lemaitre RN, Burke GL, Lyles MF, Lefkowitz D, Siscovick DS. Fish intake and risk of incident atrial fibrillation. Circulation 2004; 110: 368-373.

23) Mozaffarian D, Prineas RJ, Stein PK, Siscovick DS. Dietary fish and n-3 fatty acid intake and cardiac electrocardiographic parameters in humans. J Am Coll Cardiol 2006; 48: 478-484.

24) Den Ruijter HM, Berecki G, Verkerk AO, Bakker D, Baartscheer A, Schumacher CA, Belterman CN, de Jonge N, Fiolet JW, Brouwer IA, Coronel R. Acute administration of fish oil inhibits triggered activity in isolated myocytes from rabbits and patients with heart failure. Circulation 2008; 117: 536-544.

25) Abdukeyum GG, Owen AJ, McLennan PL. Dietary n-3 Long-chain polyunsaturated fatty acids inhibit ischemia and reperfusion arrhythmias and infarction in rat heart not enhanced by ischemic preconditioning. Nutr 2008; 138: 1902-1909.

26) Szentandrássy N, Pérez-Bido MR, Alonzo E, Negretti N, O'Neill SC. Protein kinase A is activated by the n-3 polyunsaturated fatty acid eicosapentaenoic acid in rat ventricular muscle. J Physiol 2007; 582: 349-358.

27) Zhao Z, Wen H, Fefelova N, Allen C, Guillaume N, Xiao D, Huang C, Zang W, Gwathmey JK, Xie LH. Docosahexaenoic acid reduces the incidence of early afterdepolarizations caused by oxidative stress in rabbit ventricular myocytes. Front Physiol 2012; 3: 252-258.

28) Moreno C, Macías A, Prieto A, de la Cruz A, González T, Valenzuela C. Effects of n-3 polyunsaturated fatty acids on cardiac ion channels. Front Physiol 2012; 3: 245-252.

29) Leaf A, Xiao YF, Kang JX, Billman GE. Prevention of sudden cardiac death by n-3 polyunsaturated fatty acids. Pharmacol Ther 2003; 98: 355-377.

30) Tamargo J, Caballero R, Gómez R, Valenzuela C, Delpón E. Pharmacology of cardiac potassium channels. Cardiovasc Res 2004; 62: 9-33.

31) Lai LH, Dong PS, Li ZZ, Li ZJ, Wang RX, Jiang WP. Effects of docosahexaenoic acid on sodium channel current and transient outward potassium channel current in rat ventricular myocytes. Zhonghua Xin Xue Guan Bing Za Zhi 2011; 39: 451-455.

32) Dhamoon AS, Pandit SV, Sarmast F, Parisian KR, Guha P, Li Y, Bagwe S, Taffet SM, Anumonwo JM. Kir2.x properties determine regional and species differences in the cardiac inward rectifier K+ current. Circ Res 2004; 94: 1332-1339.

33) Li GR, Dong MQ. Pharmacology of cardiac potassium channels. Adv Pharmacol 2010; 59: 93-134.

34) Dhamoon AS, Jalife J. The inward rectifier current (IK1) controls cardiac excitability and is involved in arrhythmogenesis. Heart Rhythm 2005; 2: 316-324.

35) Jalife J. Inward rectifier potassium channels control rotor frequency in ventricular fibrillation. Heart Rhythm 2009; 6: S44-48.

36) Boland LM, Drzewiecki MM. Polyunsaturated fatty acid modulation of voltage-gated ion channels. Cell Biochem Biophys 2008; 52: 59-84.

37) Pound EM, Kang JX, Leaf A. Partitioning of polyunsaturated fatty acids, which prevent cardiac arrhythmias, into phospholipid cell membranes. J Lipid Res 2001; 42: 346-351.