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Original article

Eicosapentaenoic acid reduces ischemic ventricular fibrillation via altering monophasic action potential in pigs

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ABSTRACT

Although high intake of n-3 fatty acids is associated with reduced mortality of patients with ischemic heart disease, especially reduction in sudden cardiac death (SCD), the detailed mechanisms remain to be elucidated. Thus, the present study was designed to examine whether long-term treatment with eicosapentaenoic acid (EPA), a major component of n-3 fatty acids, reduces ischemia-induced ventricular fibrillation (VF) in pigs in vivo, and if so, what molecular mechanisms are involved. Male pigs were treated with either a control chow (control group) or a control chow plus EPA (600 mg/kg/day, PO, EPA group) for 3 weeks and were subjected to myocardial ischemia for 90 min (n = 8 each) with measurement of the monophasic action potential (MAP), as a marker of ventricular electrophysiological activities. The EPA treatment significantly attenuated the occurrence of VF (control 5.1 ± 1.7 vs. EPA 1.5 ± 0.8 times/animal, $P<0.05$) and markedly reduced the mortality (control 50% vs. EPA 0%, $P<0.05$), with the attenuation of MAP duration shortening during ischemia (control −28.1 ± 3.0% vs. EPA −18.2 ± 1.4%, $P<0.05$). These beneficial effects of EPA were abolished by pre-treatment with cromakalim, a K_{ATP} channel opener (0.3 μg/kg/min, IC). Furthermore, EPA significantly inhibited the mRNA and protein expression of Kir6.2, a major component of sarcolemmal K_{ATP} channels, in both the ischemic region and non-ischemic regions. These results indicate that long-term treatment with EPA reduces ischemia-induced VF and SCD in pigs in vivo, for which attenuation of MAP duration shortening may be involved.

1. Introduction

Although the management of patients with acute myocardial infarction (AMI) has been markedly improved, more than 50% of those AMI patients still die prior to hospitalization [1]. Most pre-hospital deaths due to AMI occur within the first hour after the onset, mainly caused by ischemia-induced ventricular fibrillation (VF) but not by reperfusion arrhythmia [2]. Thus, it is critically important to develop an effective strategy to suppress ischemia-induced VF in the early phase of AMI in order to reduce sudden cardiac death (SCD).

Epidemiologic [3,4] and interventional studies [5,6] have shown that high intake of fish oil and long-chain n-3 polyunsaturated fatty acids (n-3 PUFA), including eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:5), could reduce SCD. Importantly, the GISSI-Prevenzione trial demonstrated that long-term treatment with n-3 PUFA reduces SCD after AMI [7]. However, the detailed mechanisms for the inhibitory effects of n-3 PUFA on SCD and ischemia-induced VF have not been fully elucidated.

During myocardial ischemia, rapid activation of cardiac ATP-sensitive potassium channel (K_{ATP} channel) plays an important role in ischemia-induced VF via action potential shortening and heterogeneous ventricular repolarization [8,9]. EPA possesses several beneficial effects on the pathological processes of AMI, including inhibition of thrombus formation [10] and inflammation [11] and stimulation of endothelial production of nitric oxide [12]. In addition, the acute effect of EPA on the electrophysiological properties also was reported in the previous study [13], however, the long-term effects of EPA on ischemia-induced VF and SCD in vivo remain to be examined. In the present study, we demonstrated that long-term oral treatment with EPA reduces ischemia-induced VF and SCD in pigs in vivo through the attenuation of ischemia-induced action potential shortening, for which might be mediated by suppression of myocardial K_{ATP} channels.

2. Materials and methods

The investigation conforms to 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). All procedures were performed according to the protocols approved by the Institutional Committee for Use and Care of Laboratory Animals of Tohoku University (20MdA-46).

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2.1. Animals and EPA treatment

A total of 32 domestic male pigs (2–3 month-old and weighing 20–30 kg) were randomly divided into the following 2 groups; 16-pigs were orally given EPA (600 mg/kg/day, EPA ethyl ester of purity >99%, Mochida Pharmaceutical, Tokyo, Japan) for 21 days (EPA group), and the remaining 16-pigs were fed with a standard chow alone (control group). The present dose and duration of the EPA treatment were determined based on the previous study with rabbits [14]. The 30 kg body weight animals in the control group were fed only with the regular diet including 28 g fat, whereas a total fat intake in the EPA group was assessed to be 46 g.

2.2. Fatty acid analysis

The fatty acids composition of plasma, RBC, and homogenized heart tissue extracted from the interventricular septum (IVS) (100 mg of tissue/ml of saline) was determined by capillary gas chromatography [11]. Total lipids were extracted by Folch's procedure and then fatty acids were methylated with boron trifluoride and methanol, and then methylated fatty acids were analyzed using a gas chromatograph (Shimadzu GC-17A, Shimadzu Corporation, Kyoto, Japan) and a BPX70 capillary column (0.25 mm in internal diameter × 30 m in length, SGE International Ltd., Melbourne, Australia). Tricosanoic acid, C23:0 was used as an internal standard.

2.3. Porcine model of acute myocardial ischemia

After the 3-week treatment, the animals were anesthetized with ketamine hydrochloride (20 mg/kg, IM) and sodium pentobarbital (20 mg/kg/h, IV). Surface ECG, heart rate, and arterial blood pressure were continuously monitored by a polygraph recording system (LEG1000, Nihon-Kohden, Tokyo, Japan). We inserted a 7-Fr sheath into the left carotid artery for cardiac catheterization and a 9-Fr sheath into the right carotid artery for evaluation of monophasic action potential (MAP), which is a useful marker of cardiac electrical stability [15]. Then, myocardial ischemia in the territory of the left circumflex coronary artery (LCx) was induced by inflated a coronary angioplasty balloon catheter with a lowest pressure that completely occluded distal coronary flow. Global and regional left ventricular function during ischemia was assessed by left ventriculography under occluding the LCx (n = 4 each) [16]. For pulseless ventricular tachycardia (VT) or VF, direct current (DC) shock was delivered at 200, 300 and 360J in a stepwise manner. After 90 min of myocardial ischemia, the heart tissues were immediately extracted from the inter-ventricular septum (IVS) as a non-ischemic area and from the posterior wall (PW) of the left ventricle as an ischemic area, stored under −80 °C frozen by liquid nitrogen. The extent of the risk area relative to the left ventricle was evaluated by Evans blue staining (n = 4 each).

2.4. Measurement of monophasic action potential (MAP)

To evaluate the regional action potentials in vivo, MAP was measured as a useful marker of cardiac electrical stability [15]. MAP was recorded by 6-Fr MAP catheter (EP technologies, San Jose, CA). The MAP catheter was advanced into the LV under fluoroscopic guidance and recorded the MAP at the endocardial side of the LV by the contact electrode technique. First, MAP was measured at the IVS for evaluating electrical activities in the non-ischemic area and then moved toward the PW of the left ventricle to record MAP at the ischemic area throughout the experiment. MAP signals (amplified and filtered at a frequency of 0.05 to 200 Hz) were displayed on 8-channel PowerLab data acquisition system (AD Instruments Inc., Colorado, CO) simultaneously with ECG (Supplemental Figure 1). MAP was obtained under spontaneous heart beat after placement of the catheter electrode in a position that provided continuous and adequate recordings, when a stable amplitude, smooth configuration and isopotential diastolic baseline (phase 4) persisted for at least 5 min. The QT interval, MAP duration at 90% repolarization ($MAPD_{90}$) and at 50% repolarization ($MAPD_{50}$) was measured, and collected for root square of RR interval (QTc, $MAPD_{90}$c and $MAPD_{50}$c) because these parameters highly depend on heart rate [17]. The upstroke velocity in phase 1 of MAP (dV/dt) was also calculated [18]. Chart5 (AD Instruments Inc., Colorado, CO) was used for analysis of MAP and ECG.

2.5. Intracoronary pre-treatment with cromakalim and 5-hydroxydecanoate

To evaluate the possible role of cardiac K_{ATP} channel, additional 16-pigs (n = 8, each) were pre-treated with cromakalim (Sigma-Aldrich Inc., St Louis, MO), an agent selectively opens K_{ATP} channel in the heart [19]. Cromakalim was dissolved in the vehicle (saline with polyethylene glycol and ethanol at a final concentration of <1%), selectively infused into the LCx, starting with 10 μg/kg bolus infusion at 10 min before myocardial ischemia followed by continuous infusion at 0.3 μg/kg/min until the end of the experiment. For selective drug administration into the ischemic LCx area, an infusion balloon catheter (Attendant®, Terumo Clinical Supply Co., Ltd, Gifu, Japan) was used, which enables to deliver drugs to the distal site while occluding the proximal LCx. To further evaluate the specific role of mitochondrial K_{ATP} channel, additional 8-pigs were pre-treated with 5-hydroxydecanoate (5-HD; Sigma-Aldrich Inc., St Louis, MO), an agent selectively inhibits mitochondrial K_{ATP} channel in the heart [20]. 5-HD was infused into the LCx for 45 min before myocardial ischemia followed by continuous infusion at 0.5 mg/kg/min until the end of the experiment. The present dose and duration of cromakalim or 5-HD were determined based on the previous study in dogs [21,22].

2.6. RT-PCR for cardiac K_{ATP} channels

Total RNA was extracted using RNeasy Maxi kit (QIAGEN, Hilden, Germany) and 500 ng total RNA was reverse-transcribed using QuantiTect Reverse Transcription Kit (QIAGEN). Quantitative RT-PCR was performed for the 5 components of K_{ATP} channel, including Kir6.1 (*KCN8*), Kir6.2 (*KCNJ11*), SUR1 (*ABCC8*), SUR2A and SUR2B (*ABCC9*), using a real-time detection system (Bio-Rad Lab, Hercules, CA). The following primers were used to amplify each component and GAPDH;

(a) *KCNJ9* (forward, 5′-TGTTCCGTGTGGGCGACTTA-3′, and reverse, 5′-CAAGCGGGTTATCAACAGGAATG-3′),
(b) *KCNJ8* (forward, 5′-GTGGTGGAAACCACAGGCATC-3′, and reverse, 5′-TGGTGTTGCCGAACTTGGAGTA-3′),
(c) *ABCC8* (forward, 5′-TGCCGCACGTCTTCCTACTCTTCA-3′, and reverse, 5′-CAGGATGCCCTCTGCAATCTCACA-3′),
(d) *ABCC9* (SUR2A: forward, 5′-CAGCTGAAGAATATGGTCAAATC-3′, and reverse, 5′-CTACTTGTTGGTCATCACCAAAG-3′, SUR2B: forward, 5′-GCTTCCATTGACATGGCCACAGA-3′, and reverse, 5′-GCCAAGAGGCTTTCTGGAGTGTCA-3′) and
(e) *GAPDH* (forward, 5′-TGATGGGCATGAACCATGAGA-3′, and reverse, 5′-TCCACGATGCCGAAGTTGTC-3′).

GAPDH, *KCNJ8* and *KCNJ11* were custom designed (Takara Bio Inc., Shiga, Japan), based on the sequence of *Sus scrofa* publicly available on the web site of the National Center for Biotechnology Information. The sequence of *ABCC8* and *ABCC9* were based on the previous studies [23,24]. GAPDH was used as an internal control. SYBR Premix Ex Taq™ II (Takara Bio Inc.) was used for the detection of each components and GAPDH cDNA, respectively.

2.7. Western blot analysis for cardiac Kir6.2

To study further the inhibitory effect of EPA on Kir6.2 expression, western blot analysis for Kir6.2 was performed. The extracted samples