

Prevention of ischemia-induced ventricular fibrillation by $\omega 3$ fatty acids

(fish oil/myocardial infarction/coronary heart disease/eicosapentaenoic acid/docosahexaenoic acid)

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ABSTRACT A specially prepared dog model of myocardial infarction was used to test the efficacy of the long-chain polyunsaturated fish oil $\omega 3$ fatty acids eicosapentaenoic (20:5 *n*-3) and docosahexaenoic (22:6 *n*-3) acids to prevent ischemia-induced malignant cardiac arrhythmias. The dogs had sustained a prior experimental myocardial infarction from ligation of the left anterior descending coronary artery, and a hydraulic cuff was implanted around the left circumflex artery at that operation. After recovery from that procedure the animals were tested during a treadmill exercise test. With compression of the left circumflex artery sensitive animals will predictably develop ventricular fibrillation (VF). In such prepared dogs an emulsion of fish oil fatty acids was infused i.v. over a 50- to 60-min period just before the exercise-plus-ischemia test, and the effect on development of VF was recorded. The infusion was 100 ml of a 10% (vol/vol) emulsion of a fish oil concentrate containing 70% $\omega 3$ fatty acids with free eicosapentaenoic acid and docosahexaenoic acid composing 33.9% and 25.0% of that total, respectively. Alternatively, some animals similarly received an emulsion containing 5 ml of the free fatty acid concentrate plus 5 ml of a triacylglycerol concentrate containing 65% $\omega 3$ fatty acids with eicosapentaenoic acid and docosahexaenoic acid composing 34.0% and 23.6% of that total, respectively. In seven of eight animals the infusion of the fish oil emulsion completely prevented the acute occurrence of VF in the susceptible animals ($P < 0.005$). In five of five of these animals the subsequent exercise-plus-ischemia test after a similar infusion of an emulsion in which soy bean oil replaced the fish oil fatty acid concentrates resulted in prompt development of VF. Possible mechanisms for this protective effect of $\omega 3$ fatty acids against exercise and ischemia-induced malignant arrhythmias are considered.

From their studies among the Greenland Inuits Bang *et al.* (1) suggested the possibility that the low mortality rates among the natives from coronary heart disease (CHD) resulted from their high intake of long-chain polyunsaturated fatty acids of the $\omega 3$ class derived from ingestion of large amounts of marine vertebrates in their diet. Since these pioneering observations many studies have been reported in animals and humans on the effects of ingestion of fish and fish oils on CHD. Most, but not all, of the animal studies have demonstrated beneficial effects on experimental atherosclerosis. In the laboratory the $\omega 3$ fatty acids seem to increase the production by cells of humans and animals of some antiatherogenic factors and reduce the activity of many proatherogenic factors (for review, see ref. 2). In humans the prevention of CHD mortality by $\omega 3$ fatty acid ingestion is strongly supported by epidemiologic studies (3), but there are still very few prospective, randomized, double-blind, placebo-controlled clinical trials to determine whether CHD can, in fact, be prevented by intake of $\omega 3$ fatty acids.

Furthermore, because the epidemiologic studies are based on the consumption of fish and other marine species, questions have been raised as to whether the claimed benefits may be ascribable to other components in the fish rather than to their specific content of $\omega 3$ fatty acids.

Although the focus of most of this research has been on the effect of $\omega 3$ fatty acids in preventing the atherosclerotic component of CHD, McLennan *et al.* have found that a diet high in fish oil, in contrast to saturated fat, prevents ventricular fibrillation (VF) induced by coronary artery ligation in rats with and without reflow (4) and increases the electrical VF threshold in marmosets (5). In our laboratory Hallaq *et al.* (6, 7), studying ouabain toxicity in isolated neonatal rat cardiac myocytes, demonstrated that the arrhythmogenic toxicity of high concentrations of ouabain could also be prevented by low concentrations (5 μM) of the $\omega 3$ fatty acids, eicosapentaenoic acid (20:5 *n*-3, EPA) and docosahexaenoic acid (22:6 *n*-3, DHA) but could not be prevented by $\omega 6$ arachidonic acid (20:4 *n*-6). Furthermore, the mechanism responsible for this protective effect of these $\omega 3$ fatty acids was shown to result from the prevention of the ouabain-induced rise to high toxic levels of free cytosolic Ca^{2+} within the cardiac myocyte (6). EPA and, even more effectively, DHA specifically modulate the calcium currents through dihydropyridine-sensitive L-type Ca^{2+} channels in the sarcolemma of the rat myocytes (7). This modulating effect on the L-type Ca^{2+} channels has been confirmed by Bogdanov *et al.* (8) in patch-clamp studies. Although our initial belief was that the $\omega 3$ fatty acids had to be incorporated into the phospholipids of the myocyte membranes, we found that these fatty acids, when added directly to the bathing medium together with the ouabain, still prevent the toxicity (7).

It was, therefore, the purpose of this study to investigate the effects of the acute administration of $\omega 3$ fatty acids on the induction of VF in animals known to be susceptible to sudden death. In particular, the hypothesis that the $\omega 3$ fatty acids EPA and DHA could prevent malignant arrhythmias induced by myocardial ischemia was tested by using a conscious canine model of sudden death. The present report indicates that the fish oil fatty acids will acutely prevent ischemia-induced VF in an experimentally prepared dog model.

METHODS

Mongrel dogs (14.5–18.3 kg) were used in this study. The animals were anesthetized and instrumented to measure left circumflex coronary artery blood flow and ventricular electrogram, as described (9–12). A hydraulic occluder was also placed around the left circumflex coronary artery. Finally, the left anterior coronary artery was ligated, producing an anterior wall myocardial infarction.

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Abbreviations: VF, ventricular fibrillation; CHD, coronary heart disease; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

The principles governing the care and treatment of animals as expressed by the American Physiological Society were followed at all times during this study. In addition, the procedures used in this study were approved by the Ohio State University Institutional Animal Care and Use Committee.

The studies began 3 to 4 weeks after the production of the myocardial infarction. The animals were walked on a motor-driven treadmill and adapted to the laboratory during this period. The susceptibility to VF was tested, as described (9–12). Briefly, the animals ran on a motor-driven treadmill while work load increased every 3 min for 18 min or until a criterion heart rate of 210 beats per min (70% of maximum) was attained. During the last minute of exercise, the left circumflex coronary artery was occluded, the treadmill was stopped, and the occlusion was maintained for an additional minute. The occlusion, therefore, lasted a total of 2 min. Large metal plates (11 cm in diameter) were placed across the animal's chest so that electrical defibrillation could be achieved with a minimal delay, but defibrillation was begun only after the animal was unconscious. The occlusion was immediately released if malignant arrhythmias occurred. Seven animals that developed VF during this exercise-plus-ischemia test were selected for additional studies. A long experience with this canine preparation has shown that once an animal responds with VF to this exercise-plus-ischemia test, that dog will always respond similarly with VF (9). VF was induced in one additional animal by the combination of cocaine (1.0 mg/kg i.v.) and the exercise-plus-ischemia test (13, 14).

One week later the exercise-plus-ischemia test was repeated after the following treatment. An emulsion of fish oil fatty acids in water was prepared in the following proportions: 10 ml of fish oil concentrates with 1.5 g of purified egg lecithin, 200 international units of α -tocopherol, 50 mg of butylated hydroxytoluene added to 100 ml of water containing 1.25 g of glycerol, pH 7.4 adjusted with NaOH, and the mixture was sonicated to yield a milky white and stable emulsion. A loading dose of 15–20 ml of the emulsion was injected as a bolus over 5 min; this was followed by an i.v. infusion of the remainder of the emulsion over the following 50–60 min. The exercise-plus-ischemia test was then repeated as above. One week later a second control exercise-plus-ischemia test was repeated after either saline ($n = 3$) or an I.V. infusion over 50–60 min (as with the fish oil emulsion) of 100 ml of Intralipid, a 10% lipid emulsion ($n = 5$) containing soy bean oil with only $\approx 7\%$ $\omega 3$ fatty acids as α -linolenic acid (18:3 $n-3$) (15) but containing no EPA or DHA. Cardiac function was monitored by ventricular electrocardiography and an intraventricular transducer for left ventricular pressure ($n = 3$). A pulsed Doppler flow transducer around the left circumflex coronary artery was used to confirm completeness of the coronary artery occlusion. Heart rate was averaged over the last 5 sec of each exercise level, immediately before and at the end of the 60 sec (or immediately before VF) time points during the occlusion.

Toxic effects of the emulsion, manifested by an acute respiratory distress syndrome, were encountered initially but were obviated when a more stable, purer emulsion was attained. No other adverse reactions were noted, but detailed studies of this were not done. The initial four dogs studied were infused with 10 ml of a fish oil concentrate containing 70% $\omega 3$ fatty acids with free EPA and DHA composing 33.9% and 25.0% of that total, respectively. A further four animals received an emulsion containing 5.0 ml of the same free fatty acid preparation and 5.0 ml of a triacylglycerol concentrate containing 65% $\omega 3$ fatty acids with EPA and DHA composing 34.0% and 23.6% of that total, respectively.

The fish oil free fatty acid and triacylglycerol concentrates were provided by Pronova-Biocare (Lysaker, Norway). The

egg lecithin was obtained from Avanti Polar Lipids, and the Intralipid was from Clintec Nutrition (Deerfield, IL). The other chemicals used were all reagent grade.

The data were analyzed by using a two-factor (drug \times occlusion) analysis of variance (ANOVA) for repeated measures. When the F -ratio was found to exceed a critical value ($P < 0.05$), Scheffe's test was used to compare the means. The effect of the $\omega 3$ fatty acids on VF was determined by using a χ^2 test with Yate's correction for continuity. All data are reported as the mean \pm SEM. Cardiac arrhythmias were analyzed at a paper speed of 25 mm/sec.

RESULTS

Representative ventricular electrogram recordings obtained from the same animal before and after pretreatment with the $\omega 3$ fatty acids are displayed in Fig. 1. In agreement with previous studies (9–12) ventricular flutter (which degenerates to VF) was reproducibly induced with each presentation of the control (both saline and Intralipid infusions) exercise-plus-ischemia tests. The cocaine exercise-plus-ischemia test induced a similar response. Data for all animals that developed VF have, therefore, been combined. The average time to the onset of VF was 55.1 ± 2.8 sec (range from 42 to 63.4 sec): four animals developed VF shortly after the treadmill stopped, whereas four animals developed malignant arrhythmias while running. In contrast, $\omega 3$ fatty acids prevented ventricular arrhythmias in seven (includes the cocaine-induced VF) of the eight (87.5%) susceptible animals ($\chi^2 = 9.14$, $P < 0.005$).

$\omega 3$ fatty acids evoked significant reductions in heart rate (drug effect $F = 7.86$, $P < 0.05$) both before (control, 211.4 ± 16.5 versus fish oil, 163.6 ± 17.0 beats per min) and during the coronary occlusion (control, 227.4 ± 16.1 versus fish oil, 164.9 ± 22.8 beats per min). The i.v. infusion of the $\omega 3$ fatty acids reduced heart rate in six of the eight animals and increased heart rate in two animals; both animals with the increased heart rate were still protected by the infusions of $\omega 3$ fatty acids. In a similar manner, P wave–R wave intervals significantly ($P < 0.01$) increased after the infusion of $\omega 3$ fatty acids (control, 91.7 ± 5.5 versus fish oil, 108.9 ± 6.8 msec); second-degree (2:1) atrio-ventricular block was, in fact, induced in four of the eight animals.

DISCUSSION

The present study demonstrates that the long-chain polyunsaturated $\omega 3$ fatty acids concentrated from fish oil can prevent ischemia-induced VF in animals known to be susceptible to sudden death. Specifically, the i.v. infusion of an emulsion containing predominantly EPA and DHA prevented VF induced by the combination of ischemia and submaximal exercise in seven of eight dogs (including one animal tested with cocaine). Thus, we report a statistically significant prevention of VF in conscious animals by the acute i.v. infusion of $\omega 3$ fatty acids.

McLennan and associates (4) reported that dietary $\omega 3$ fatty acids (tuna fish oil) reduced the incidence and severity of arrhythmias in anesthetized rats, preventing VF during both coronary artery occlusion and reperfusion. They also reported that the VF threshold (the amount of current required to induce VF) of adult marmoset monkeys was elevated during ischemia by a supplement of fish oil (3% of energy of a total dietary fat intake of 31% of energy) (5). The effects of the acute administration of $\omega 3$ fatty acids on arrhythmias were not investigated in their studies.

A growing body of evidence suggests that an accumulation of myocardial cytosolic calcium during ischemia plays a critical role in the genesis of malignant arrhythmias (14, 16–18). It is probable that superimposed impulses of calcium

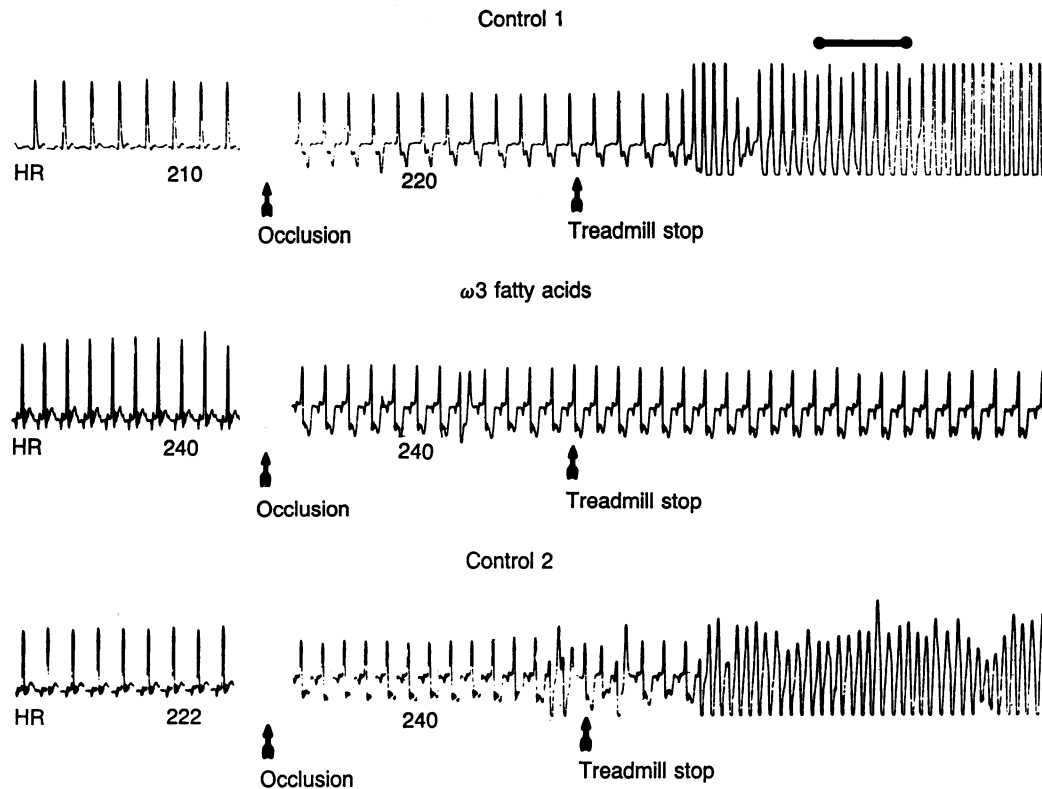


FIG. 1. Representative ventricular electrograms from the same animal with and without treatment with i.v. ω 3 fatty acids. Control 1: exercise-plus-ischemia test 1 week before the ω 3 fatty acid exercise-plus-ischemia test. Control 2: exercise-plus-ischemia test repeated after a 60-min infusion of lipid emulsion derived from soy bean oil (Intralipid) lacking long-chain ω 3 EPA or DHA but containing 7% ω 3 α -linolenic acid. The second control was done 1 week after the ω 3 fatty acid exercise-plus-ischemia test (middle electrogram strip). Dark bar, 1.0 sec; paper speed, 25 mm/sec; HR, heart rate (beats/min).

influx via L-type calcium channels are required to induce the VF, as reported by Merrill *et al.* (19) and du Toit and Opie (20). In related studies, Billman and colleagues (9, 11, 12) demonstrated that several calcium channel antagonists prevented VF induced by exercise plus ischemia. Conversely, the calcium channel agonist, Bay K 8644, induced VF in animals resistant to the development of malignant arrhythmias (9). In contrast, the plant alkaloid ryanodine, which disrupts calcium release from the sarcoplasmic reticulum, failed to prevent malignant arrhythmias despite large reductions in cytosolic calcium levels (21). They concluded "that calcium flux across the sarcolemma may contribute more to the development of VF than excess calcium release from the sarcoplasmic reticulum." Calcium overload has also been implicated in the cocaine-induced arrhythmias (22). In this regard Billman reported that calcium channel antagonists (13), but not ryanodine (14), prevented malignant arrhythmias induced by the combination of cocaine and an exercise-plus-ischemia test.

There seem to be similarities in the mechanisms that may be involved in the mechanisms of VF induced by ouabain toxicity and by ischemia. With ouabain toxicity there is also an associated overload of cytosolic free calcium levels in the cardiac myocytes. This overload results from reversal of the $\text{Na}^+/\text{Ca}^{2+}$ antiport system, which overloads the cardiac myocytes with calcium (23). Dihydropyridine antagonists, which affect specifically the L-type calcium channels, the same channels that are modulated by the ω 3 fatty acids (7, 8), will prevent ouabain toxicity (7). It may be that the pulsatile influx of calcium via L-type calcium channels superimposed on the high cytosolic levels from the reversal of the $\text{Na}^+/\text{Ca}^{2+}$ antiport system is essential for the development of ouabain-induced VF. This mechanism may be the common denominator that allows the ω 3 fatty acids to prevent both the

ouabain and the ischemia-induced VF. Consistent with this reasoning is the observation that the ω 3 fatty acid infusion also significantly prolonged P wave-R wave intervals. The P-R interval, which reflects atrioventricular nodal conduction, is critically dependent on calcium influx and, as a result, is significantly increased by the infusion of calcium channel antagonists, such as verapamil or diltiazem (11).

The dog is very unpredictable regarding its likelihood to fibrillate when one or more coronary arteries are ligated in a normal heart. By using the specially prepared animals described by Billman and colleagues (9–12), we were able to achieve a statistically significant result with many fewer animals than with unprepared dogs. He has developed a highly reliable and reproducible animal model in which to test antiarrhythmic agents. The VF was highly reproducible in the susceptible animals—that is, VF could be repeatedly induced on each presentation of the test of exercise plus ischemia (once a week for up to 8 weeks, the longest period studied). Thus, unless some effective intervention is applied, a susceptible animal will remain susceptible to VF throughout the course of the study.

It was noted that the ω 3 fatty acids also reduced heart rate and could, therefore, indirectly protect against ischemic arrhythmias by lowering cardiac metabolism and allowing more time for the high cytosolic calcium levels to be cleared. The contribution, if any, of reductions in heart rate to the cardioprotection noted for the ω 3 fatty acids was not evaluated in the present study. We noted, however, that heart rate was not altered in two dogs, yet the ω 3 fatty acid emulsion still prevented VF. Heart rate effects alone, therefore, may not explain the protection noted for the fish oil preparation.

We have not yet examined the effects of EPA or DHA on ion channels other than the L-type calcium channels in heart cells. Apparently long-chain, polyunsaturated fatty acids—

both arachidonic acid and DHA—directly inhibit the major cardiac, voltage-sensitive, rectifier potassium channel (Kv 1.5) of heart cells, as demonstrated by patch-clamp studies (26). This fact may explain the partial reduction of VF noted by McLennan *et al.* (4) by dietary sunflower seed oil compared with essentially complete prevention by fish oil (tuna fish oil) of ischemia-induced VF in rats. Arachidonic acid blocks the potassium channel, whereas DHA affects both the potassium and the L-type calcium channels.

Studies are needed to clarify further the means by which EPA and DHA protect the heart *in vivo* from ischemia-induced VF. Whether this protective effect will occur in other species, including humans, remains to be seen. The earlier studies of McLennan *et al.* (4, 5) demonstrated protection by fish oil of rats from ischemia-induced VF and increased VF threshold in marmosets in chronic feeding experiments; these results encourage the likelihood of a protective effect in humans. Thus far, there have been no studies reported in humans to test for a possible antiarrhythmic action of ω 3 fatty acids in ischemia-induced VF. The Death and Reinfarction Trial (24) is of particular interest, however, in this regard. In that study some 2000 men who had just suffered a myocardial infarction were randomized as to either (i) the advice to eat oily fish two to three times weekly or (ii) no advice regarding fish intake. At the end of only 2 yr there was a 29% reduction in mortality among those advised to eat fish as compared with the control group. But interestingly there were no differences in new cardiac events; if one had a myocardial infarction, those ingesting fish were simply less likely to die than the controls who were not advised to eat fish. The mortality difference between the two groups, furthermore, began to appear at 2 months into the study. This result suggests that the difference in mortality may have resulted from the prevention of VF in the cohort advised to eat fish. In the U.S.A. of the half-million deaths annually from myocardial infarctions, 300,000, some 60%, are "sudden deaths" (25), which are almost all caused by VF. Thus the ω 3 fatty acids from marine sources have the potential for an important public health role in the prevention of deaths from coronary heart disease, which still is the leading cause of mortality in the United States and other Western industrialized countries and a leading cause of morbidity and health costs as well.

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