Myocardial protection by ischemic preconditioning: The influence of the composition of myocardial phospholipids

S. Al Makdessi, M. Brändle, M. Ehrt, H. Sweidan and R. Jacob
Physiologisches Institut II, Universität Tübingen, Germany

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Abstract

It was the aim of this study to investigate (1) whether preconditioning modifies the fatty acid (FA) composition of myocardial phospholipids (PL), (2) whether a previous modification of membrane PL composition by the administration of coconut oil or fish oil influences the preconditioning, and (3) to compare the protective effects of preconditioning to those of dietary fish oil. To this end, three groups of rats were given during 10 weeks either a standard diet, or a standard diet +10% coconut oil, or a standard diet +10% fish oil. The preconditioning was performed in situ in the anesthetized open-chest rats by 2 cycles of 3 min left anterior descending coronary artery occlusion and 10 min reperfusion. It was followed by a 40 min ischemia and a 60 min reperfusion. ECG was recorded and used for the continuous count of the salvos of extrasystoles, ventricular flutter and fibrillation. These rhythm disturbances were subsequently added and evaluated as total arrhythmias. The FA of tissue PL were analyzed in a sample of the ischemic zone the size of which was determined by means of malachite green.

Coconut oil diet (rich in saturated FA) modified slightly the myocardial PL by increasing oleic acid and decreasing linoleic acid and resulted in the highest incidence of arrhythmias. Fish oil diet had the opposite effect in modifying drastically the PLFA (replacement of the n-6 FA by the n-3 FA) and minimizing significantly the arrhythmias in comparison with the standard diet group. The antiarrhythmic effect of preconditioning could be observed only after coconut oil had been administered and was not accompanied by a modification of PL composition. The reduction of arrhythmias in this case was comparable to that observed under fish oil administration with and without preconditioning. The size of the ischemic zone remained unchanged.

We conclude that the protection by ischemic preconditioning is not mediated by the modification of the composition of heart PL, and that the n-3 FA diet had such a protective effect that no additional protection could be supplied by ischemic preconditioning. (Mol Cell Biochem 145: 69-73, 1995)

Key words: preconditioning, coconut oil, fish oil, size of the ischemic zone, incidence of arrhythmias, myocardial phospholipids, saturated fatty acids, n-3 fatty acids

Abbreviations: 12:0 – lauric acid; 14:0 – myristic acid; 16:0 – palmitic acid; 16:1 n-7 t – trans-palmitoleic acid; 16:1 n-7 c – cis-palmitoleic acid; 18:0 – stearic acid; 18:1 n-9 – oleic acid; 18:1 n-7 – vaccenic acid; 18:2 n-6 – linoleic acid; 18:3 n-3 – α-linolenic acid; 20:3 n-6 – dihomo γ-linolenic acid; 20:4 n-6 – arachidonic acid; 20:5 n-3 – eicosapentaenoic acid (EPA); 22:4 n-6 – eicosatetraenoic acid; 22:5 n-3 – docosapentaenoic acid (DPA); 22:6 n-3 – docosahexaenoic acid (DHA); BHT – butylated hydroxytoluene

Introduction

One of the numerous hypotheses which aimed to investigate the mechanism(s) of the protective effects of myocardial preconditioning was the membrane lipid hypothesis [1], according to which the stress caused by the preconditioning...
would lead to a favourable rearrangement of the fatty acids (FA) of membrane lipids. The polyunsaturated fatty acids (PUFA) would be particularly concerned as they are able to modify the physical properties of the membrane [2].

The investigation carried out by Jones and coworkers [1] in the pig has focused on two saturated (16:0, 18:0), one monounsaturated (18:1 n-9), and two polyunsaturated (18:2 n-6, 20:4 n-6) FA. The preconditioning increased the content of all of these FA into membrane phospholipids (PL). Thus, it seemed of particular interest to study whether the n-3 PUFA could be rearranged in the same way, and to check whether another animal species, in our case the rat, would react similarly.

Another cardioprotective intervention is the administration of oils rich in PUFA, such as corn, linseed, and fish oils [3-5]. The most efficient was found to be fish oil, which modifies the composition of membrane PL [6-8] and increases both n-3/n-6 ratio and the double bond index (DBI) [8, 9].

On the other hand, hydrogenated coconut oil contains only trace amounts of PUFA and a high proportion of saturated FA. Its intake caused minor modifications in the composition of membrane PL [8], but was reported to increase the cholesterol and PL content of the membrane [10]. Although there is a lack of information concerning the cardiovascular effects of coconut oil administration, it is generally believed that the saturated FA supplementation could unfavourably modify the electrophysiological properties of the heart [11].

On the basis of these observations it was the aim of this study to investigate:

1. whether the preconditioning modifies the composition of membrane PL
2. whether a previous alteration of membrane PL composition (either by coconut oil or by fish oil administration) could influence the preconditioning
3. to compare the protective effects of ischemic preconditioning on the one hand and that of the dietary application of n-3 FA on the other.

### Materials and methods

#### Animal feeding

Forty six two month old male Wistar rats were divided into 3 feeding groups: The first (n = 19) was given a standard chow diet (<1% w/w, Altromin GmbH and Company KG, Lage/Germany). The second group (n = 16) was given a standard diet enriched with 10% fish oil (sardine oil, J.C. Martens, Bergen/Norway), and the third group (n = 11) was given a standard diet enriched with 10% hydrogenated coconut oil (Union Deutsche Lebensmittelwerke GmbH, Hamburg/Germany).

The animals were housed at constant temperature and kept on a 12 h light-dark cycle. Fresh food was given 3 times a week. Coconut oil is characterized by a high content in saturated FA (>60%), and fish oil by the presence of n-3 PUFA, 20:5 n-3 (14.5%) and 22:6 n-3 (14.6%) (the detailed composition of these oils is given in ref. [8]).

#### Preconditioning protocol

After a 10 week feeding period the rats were randomly divided into preconditioned (P) and non-preconditioned, or control (C) animals. They were anesthetized intraperitoneally with pentobarbital (30 mg/kg body weight), artificially ventilated, and the chest was opened. After a short equilibration period (10 min), the heart of the P group animals was preconditioned in situ by means of 2 cycles of 3 min left anterior descending coronary artery occlusion - 10 rain reperfusion. This was followed by a 40 min ischemia and a 60 min reperfusion. The ischemia of the (C) group animals was induced 36 min after opening the chest.

ECG was recorded continuously. At the end of the experiment, 5 ml of a 0.4% (w/v) solution of malachite green were injected for the assessment of the size of the occluded zone (area of risk), and a sample of this zone was taken for the analysis of heart PL and stored at -65°C.

#### Evaluation of arrhythmias

In each experiment the arrhythmias were counted taking into consideration only the malignant ventricular tachyarrhythmias in which the segment QRS was abnormal. This type of rhythm disturbances comprised:

- salvos of extrasystoles (more than 4 consecutives extrasystoles with the maximal duration of 30 sec under the threshold frequency of flutter),
- ventricular flutter and fibrillation (defined according to Walker et al., ref. [12]).

The arrhythmias were estimated in each experimental group as relative values by summing up the total counts of arrhythmias and dividing it by the number of animals:

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\text{Incidence of arrhythmias} = \frac{\text{sum of the arrhythmias observed on all animals of each group}}{\text{number of animals}}
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Then, the results (which are given as mean values) were listed in a contingency table and statistically treated according to Poisson