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Effects of omacor® on left ventricular remodelling consecutive to post myocardial infarction special issue-myocardial infarction*

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ABSTRACT

Ventricular remodelling is the main trigger of the development of heart failure. Therefore, the reduction of structural remodelling is known to prevent the development of heart failure. The aim of the present study was to investigate the effects of OMACOR®, a well known mixture of EPA and DHA in an experimental model of heart failure induced by occlusion of left descending coronary artery and the reperfusion within 2 months. After a long term treatment of 2 months; OMACOR® (100 mg/kg) statistically significantly reduced the expansion of infarcted zone (35% ± 4%, P < 0.05, n = 9, versus 45% ± 3% in the vehicle group). The phosphorylation of Cx43 as biomarker of the cardiac remodelling was visualised by immunofluorescence in rat's heart at the end of the study. In the vehicle-infarcted group, a significant de-phosphorylation of Cx43 was observed (8.2 ± 1.0 u.a, n = 8 compared to 11.8 ± 1.3 u.a in the sham group, n = 9) confirming a remodelling process in the infarcted group. In the group treated with OMACOR®, the de-phosphorylation of Cx43 was no longer observed compared to the sham group (16.4 ± 2.9 u.a, n = 9, NS). The present results demonstrate that a long term treatment with OMA-COR® reduced the infarcted size in experimental models of heart failure and that these anti-remodelling effects are due at least in part by re-synchronizing the gap junction activity.

Keywords: Left Ventricular Remodelling; Myocardial Infarction; OMACOR®

1. INTRODUCTION

Occlusion of the circumflex coronary artery results in extensive myocardial necrosis and fibrosis, with a decrease in myocardial contractility and function [1-3]. Following infarction, the myocardium undergoes a prolonged remodelling process that induces widespread structural changes, with the ventricle becoming considerably stiffer and less compliant [4,5]. Histologically, the most prominent changes are myocyte death and scar formation in the infarcted myocardium. The remodelling process is not limited to the infarct area. Changes in noninfarcted myocardium include myocyte hypertrophy, apoptosis, fiber disarray, angiogenesis, and an increase in interstitial collagen, all of which can eventually lead to death from heart failure.

OMACOR®, one of the polyunsaturated fatty acid compounds, has been shown to reduce cardiovascular-related morbidity and mortality in clinical trials [6,7] and has been demonstrated to impact cardiac remodelling, including hypertrophy [8,9]. However, the beneficial effects of OMACOR® on coronary artery disease are not limited to its triglyceride-lowering function but involve various pleiotropic effects on cardiac function including reduction of arrhythmias, inhibition of cellular proliferation and migration, anti-inflammatory effects, and improvement of endothelial function [10]. Despite the widespread clinical use of OMACOR® for hypertriglyceridemia and prevention of coronary artery disease, data are lacking on the effects of OMACOR® on clinical outcome in heart failure secondary to myocardial infarction (MI). Thus, the role of OMACOR® in heart failure due to MI remains controversial. Therefore, the purpose of this study was to determine whether administration of OMACOR® during the peri-infarct period attenuates the progressive LV chamber dilatation and contractile dysfunction in a rat model of MI.

2. METHODS

2.1. Preparation of Animals

The experiments were carried out according to French

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law and the local ethical committee guidelines for animal research.

Male Sprague-dawley rats weighing 220 - 300 g at the date of the experiments were purchased from Iffa Credo (France). They were housed in the Centre de Recherche Pierre Fabre animal facilities for at least two weeks before use. Throughout this period, they had free access to food and drinking water. The animal house was maintained on a 12-h light/dark cycle (lights on at 7 a.m.) at an ambient temperature of 20˚C ± 2˚C.

2.2. Surgical Model of Myocardial Infarction

The rats were anesthetized using Isoflurane 3% on O_2. Then, the animals were intubated and ventilated at 60 respirations/min (2.5 ml/respiration, Ventilator model 683, Harvard Apparatus, HOLLISTON, MA, USA) while anesthesia was maintained. Body temperature was maintained at 37˚C by a heating pad (Homeothermic blanket control unit, Harvard Apparatus, HOLLISTON, MA, USA). A left thoracotomy was performed and a silk suture (4.0) was placed around the left coronary artery ~1 mm from its origin. Both ends of the silk thread were passed through a polyethylene tube. The left coronary artery was occluded by pressing the polyethylene tube against another polyethylene tube placed on the heart. After 30 min of ischemia, the polyethylene tube was removed to initiate a definitive reperfusion phase. Then, the thorax was closed and the animals regained consciousness 1 hour after the end of the ischemia. Finally, the reperfusion was performed for 2 months in conscious animals. Then, OMACOR® or vehicle (olive oil) was daily administrated orally by gavage using a single administration of 100 mg/kg.

2.3. Histological Analysis and Immmunochemistry

Immediately after the sacrifice, the heart was rapidly excised and fixed with AFA (alcohol, formaldehyde, acetate) for 1 - 4 days. The ventricles were cut into five cross-sectional samples of 2 mm each. The five regions were then processed into paraffin with an automated tissue processor. The samples were then embedded into fresh paraffin with the apical side down. From the third block of tissue, a 3 µm section was cut and was used for Masson staining. The stained slide was then hydrated with distilled water and then incubated twice with 100% ethanol (for 1 min each).

Masson stainings were used to quantitate interstitial collagen volume fractions as well as infarct sizes using a video image analysis system (LEICA QWIN, LEICA Imaging Systems Ltd., Cambridge, England). A color video camera (DXC-390P color videocamera, SONY, Paris, France) relayed the image to a computer through LEICA analysis software application. The following parameters were measured (in mm): septal wall, left ventricular free wall, endocardial and epicardial left ventricular circumference and endocardial and epicardial infarcts. The green color extraction was used to quantitate interstitial collagen. The equation used to calculate infarct size was the following: percent infarct of left ventricle = [epicardial infarct (in mm) + endocardial infarct (in mm)]/[LV epicardial circumference (in mm) + LV endocardial circumference (in mm)] × 100 (Sandmann *et al.*, 2001).

The phosphorylation of connexion 43 (Cx43) at intercalated disks of atrial myocardium was determined by immunofluorescence. After the sacrifice, the atria were collected and immediately immerged in Formalin solution (Sigma-Aldrich, HT50-1-2) for 10 - 15 minutes at room temperature. They were then washed three times for 10 minutes in tyrode buffer, mounted in embedding medium (Miles), frozen in isopentane precooled in liquid nitrogen, and stored at –80˚C. Immunostaining was performed on 7-µm-thick cross-sections. Tissue sections were permeabilized and saturated with 0.5% Triton X-100, 1% BSA and 10% of goat, chicken and human serum in phosphate buffered saline (PBS) for 60 minutes. They were then labeled with mouse antibody to α-actinin (1:400, Zymed) or rabbit phosphospecific antibodies to P-Ser368-Cx43 (1:50, Life Technologies) in PBS containing 1% BSA, 0.5% Triton X-100 and 3% of goat, chicken and human serum for at least 2 h at room temperature. After washing in PBS, Alexa 488-conjugated chicken anti-mouse IgG and/or Alexa 594-conjugated goat anti-rabbit IgG (1:400, Life technologies) were added with 1% BSA and 3% of goat, chicken and human serum in PBS for 1 hour. After washing in PBS, coverslips were mounted with Dako fluorescent mounting medium. For the quantification of Cx43 phosphorylation, the images were captured with Olympus BX 50 microscope and DP50 camera with magnification 200 and a similar time exposure for all the atrial cross-sections studied. Treatment of images and quantification of phospho-Cx43 labeling at the intercalated disks were performed with NIH Image J software program. The mean fluorescence intensity was counted on 10 intercalated structures by field and on 6 fields for each rat.

Images of **Figure 1(a)** were captured at magnification 600 and similar time exposure with an Olympus IX 50 microscope and a Roper Scientific camera. Images were automatically collected at 0.2 µm *Z*-intervals with a piezoelectric translator (PIFOC, Karlsruhe/Palmbach, Germany) driven by Metamorph Software (Universal Imaging Corp., Downingtown, PA). Each *Z*-series was deconvoluted automatically using a measured Point Spread Function and an adapted constrained interactive de-convolution algorithm. Treatment of images was performed with the NIH Image J software program. Sets of three consecutive z-images were compiled and treated with

similar thresholds for each condition.

2.4. Statistical Analysis

Statistical analysis of data was performed using SPSS® software version 16.0, $P < 0.05$ was considered statistically significant, and Bonferroni's *post hoc* test was used where appropriate. Continuous data were expressed as mean ± S.E. mean, and comparisons between treatment groups were made with one-way ANOVA. The incidence of AF was compared with Fisher's exact test.

3. RESULTS

3.1. Infarcted Ventricular Histology

Figure 1(a) shows typical ventricular stained slides obtained 2 months after myocardial infarction. As expected, no infarction and no collagen deposition were detected in all sham-operated animals (**Figures 1 (a)-(c)**). The vehicle exhibited infarct expansion, late-phase ventricular dilation and fibrosis (green staining) of viable myocardium, which is consistent with known post myocardial infarct tissue remodelling (**Figure 1(a)**). Indeed, the percentage of the infarcted left ventricle was 45% ± 3% (n = 7; **Figure 1(c)**). The collagen deposition in this group was largely increased (22 ± 4 mm^2), and the free wall thinning induced a significant reduction of thickness ratio (0.63 ± 0.05). OMACOR® (100 mg/kg) statistically significantly reduced the expansion of infarcted zone (35% ± 4%, $P < 0.05$, n = 9, versus 45% ± 3% in the vehicle group; **Figure 1(c)**). Despite a large tendency, OMACOR® failed to significantly reduced scare collagen deposition at the late time point (20 ± 2 mm^2, NS, **Figure 1(b)**).

3.2. Cardiac Tissue Remodelling

Phosphorylation of Cx43 as biomarker of the cardiac remodelling [11] was visualised by immunofluorescence in rat atria at the end of the study and quantified using the image J software. In the olive oil-infarcted group (vehicle), a significant de-phosphorylation of Cx43 was observed as shown in **Figure 2** (8.2 ± 1.0 u.a in the olive oil group, n = 8 compared to 11.8 ± 1.3 u.a in the sham group, n = 9) confirming a remodelling process in the infarcted group. In the infarcted group treated with OMACOR®, the de-phosphorylation of Cx43 was no longer observed compared to the sham group (16.4 ± 2.9 u.a, n = 9, NS, **Figure 2**). Similarly, the phosphorylation of Cx43 was statistically significantly restored between the olive oil group and OMACOR® groups.

Collectively, these data indicate that the marked dilation of the heart cavities that occurring in the olive oil group following 2 months after infarction and reperfusion was markedly reduced by a daily oral treatment with OMACOR® (100 mg/kg).

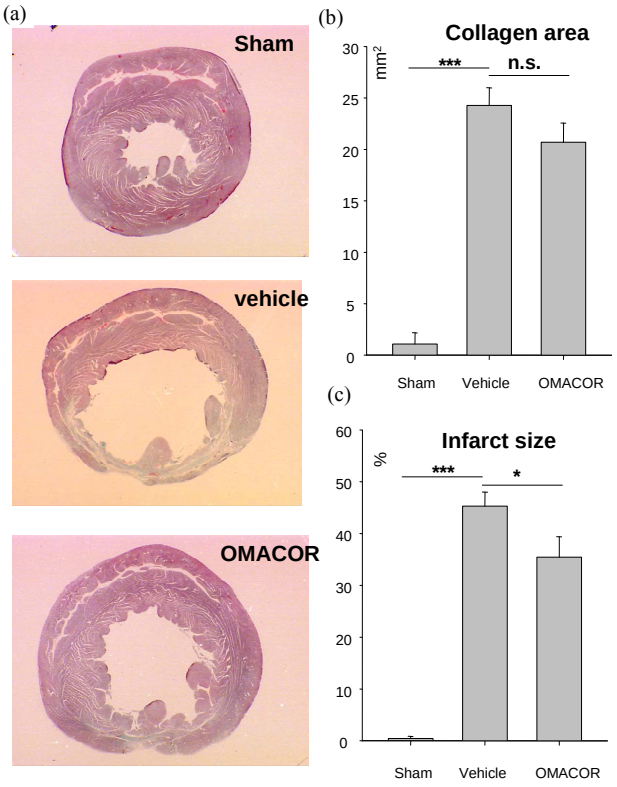

Figure 1. (a) Ventricular transaxial plane showing Masson staining of a ventricular slice after 30 min left-descending coronary occlusion and 2 months of reperfusion. Upper, Sham operated rat. Middle, vehicle-treated rat (olive oil). Lower, OMA-COR® -treated rat (100 mg/kg). The viable zone is red, the ischemic zone is white, and the collagen is green. Bar graph showing the collagen area (b) and the infarcted zone (c) in the sham operated group, in the presence of vehicle (olive oil), and OMACOR®. Data are means ± SEM. $^{*}P < 0.05$ and $^{***}P < 0.001$.

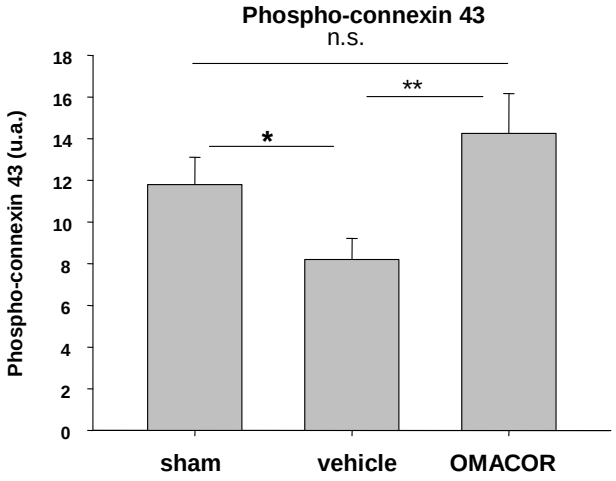

Figure 2. Bar graph showing the left atria size quantified by echocardiography (a) posphorylation of Cx43 quantified by immunostaining (b) in the sham operated group, in the presence of vehicle (olive oil), and OMACOR®. Data are means ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$. n.s: non-significant.

4. DISCUSSION

The key finding of this study was that a 2-month treatment with OMACOR® led to a significant reduction in left ventricular remodelling implicated in the development of heart failure. To the best of our knowledge, no prior studies have demonstrated the long term effects of OMACOR®, alone on all of these parameters of heart founction. In the present rat model of ischemia-induced heart failure, OMACOR® (100 mg/kg) reduced the expansion of the ventricular infarcted zone which was associated with a decrease of the heart remodelling and of the dephosphorylation of Cx43.

The cardioprotective effects of n-3 PUFA appear to be due not through a single mode of action but to a synergism between multiple, intricate mechanisms that involve TG lowering, anti-inflammatory, inflammation-resolving, regulation of transcription factors and gene expression, membrane fluidity and antiarrhythmic and antithrombotic effects [12,13]. Both EPA and DHA components of OMACOR® have similar yet very distinctive cardioprotective properties. Only DHA seems to decrease blood pressure, heart rate and the number of total and small dense LDL particles. DHA also has higher potency to regulate the activity of several transcription factors than EPA [12,13]. The present results demonstrate that OMACOR® (100 mg/kg) reduced the infarct size and induced a significant reduction of the ventricular dilation for 2 months after myocardial infarction. This cardioprotection mediated by OMACOR® was associated with a partial reduction of the collagen scar suggesting that the compound could preserve the remodelling of heart tissue consecutively to myocardial infarction. Our findings that OMACOR® reduces left ventricular dilation after myocardial infarction are in agreement with previous findings demonstrating that PUFAs reduce heart failure induced by myocardial infarction in dog [14], as well as in rat with left ventricular pressure overload [13]. These protective effects of OMACOR® on post myocardial infarction induced ventricular dysfunction are associated with a decrease of myocardial remodelling and alterations of Cx43 phosphorylation. Thus, two months after surgery, the infarcted rats had a left ventricular dysfunction and an enlarged and fibrotic left ventricle. It has been previously shown that regression of the heart remodelling in treated myocardial infracted rats was associated with re-phosphorylation and assembly of organized gap junction [11]. Thus, in the vehicle group, the large proportion of Cx43 was non-phosphorylated. Because the phosphorylation sites regulate channel properties, assembly and targeting in junctional plaques [15], the dephosphorylated Cx43 from remodelling heart is responsible for depressed cell-cell coupling [11]. Therefore, the reduction of a higher amount of non-phosphorylated Cx43 by OMACOR® and the redistribution of Cx43 suggest a re-organisation of junctional areas in heart tissue. Collectively, this cardioprotection of OMA-COR® against the dephosphorylation of Cx43 and the prevention of the atria and ventricle dilations certainly contribute to cardioprotective properties against structural remodelling-induced heart failure.

It is becoming increasingly clear from clinical and animal studies that OMACOR® alters cardiac membrane phospholipid fatty acid composition, decreases the onset of new HF, and slows the progression of established HF [6, 7]. This effect is associated with decreased inflammation and improved resistance to arrhythmia incidence. That said, there has yet to be a definitive clinical trial with an appropriately high dose of OMACOR® (>3 g/d) or comparing DHA to EPA in established HF. Definitive information on the optimal dose of OMACOR® is not available; thus additional clinical trials are warranted.

In summary, OMACOR® is a potent mixture of EPA + DHA with a high potential for the treatment of heart failure induced myocardial infarction. Indeed, the present results demonstrate that a long term treatment with OMACOR® reduces the ventricular dilation and the infarcted size in experimental models of heart failure. The present study demonstrates that these anti-remodelling effects are due at least in part by resynchronizing the gap junction activity beside the well-established cardioprotective mechanisms of the PUFAs.

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