Borage oil attenuates progression of cardiac remodeling in rats after myocardial infarction[1]

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ABSTRACT

PURPOSE: To investigate the effects of Borage oil on cardiac remodeling after myocardial infarction (MI).

METHODS: Male Wistar rats underwent ligation of the left coronary artery and divided into three groups: MI (control), BO-18 (18 mg/kg of borage oil) and BO-180 (180 mg/kg of borage oil). After seven days, heart was arrested in diastole and processed for histological evaluation of: MI size, LV dilation, myocyte hypertrophy, inflammatory infiltration and fibrosis in MI region and in remote region. The relative weight of the lung was used as a marker of heart failure. The MI size was comparable among groups.

RESULTS: Compared to control, BO treated groups showed lower weight of heart and lungs, reduced LV dilation and myocyte hypertrophy. Hemodynamic measurements were comparable. The treatment attenuated the inflammatory infiltration and fibrosis in remote myocardium.

CONCLUSION: Borage oil attenuates progression of cardiac remodeling after myocardial infarction and congestive heart failure.

Key words: Myocardial Infarction. Ventricular Remodeling. Fatty Acids, Omega-6. Rats.

Introduction

Myocardial infarction was the main cause of death 2012 in world and will the leading cause of death by the year 2030[1]. Dietary and nutritional aspects have been studied to reduce cardiovascular risk, in especially to ideal intake of polyunsaturated fatty acids omega-3 and omega-6. The omega-3 has a modulatory role in inflammation and has been used for the prevention of cardiovascular disease[2]. In contrast, the role of omega-6 in inflammation has been contradictory, showing pro-inflammatory effects or anti-inflammatory. The increase in availability of one or another may change the preference of the enzyme and the final synthesis of prostaglandins and leukotrienes[3-5].

Gamma linolenic acid, omega-6 may be synthesized from linoleic acid by the direct action of delta-6-desaturase enzyme or which can be obtained from the diet. Gamma-linolenic acid is elongated by elongase dihomo-gamma-linolenic acid. The dihomo-gamma-linolenic acid acts directly in the inflammatory response or can be metabolized to anti-inflammatory prostaglandins by cyclooxygenase. The dihomo-gamma-linolenic acid may also be subjected to a further desaturation delta-5 desaturase for the production of arachidonic acid which is metabolized to prostaglandins and leukotrienes proinflammatory cyclooxygenase and lipoxygenase[6]. The borage oil intake produces acid availability dihomo-gamma-linolenic, resulting in increased formation of inflammatory prostaglandins with anti-act directly on the modulation of lymphocytes, macrophages, interleukins and tumor-necrosis factor alpha secretion[7-9].

In the initial phase of myocardial infarction, an intense inflammatory process that characterizes cardiac remodeling it refers to cellular, molecular, and interstitial changes manifested clinically as changes in size, shape and function of the heart. The purpose of modulating cardiac remodeling and ventricular function to keep systemic circulation and physiological process, however progressive myocardial remodeling process can lead to decompensation worse prognosis and subsequently death[10].

The American Heart Association[11] does not recommend anti-inflammatory drugs after myocardial infarction. Experimental and clinical studies show that the use of anti-inflammatory drugs after myocardial infarction reduces inflammation and inhibit cardiac remodeling. Direct interference in inflammation promoted the formation of scar inefficient in infarcted myocardium wall and hence the development of ventricular aneurysm and rupture. The attenuation of collagen deposition in remote regions carried out to systolic dysfunction, poor prognosis and increased mortality[11-13].

Decreased coronary perfusion pressure that occurs immediately after myocardial infarction increases the susceptibility to injury subendocardial: myocyte necrosis, inflammatory response and fibrosis; and these are related to ventricular dilatation and hypertrophy remaining myocytes. In the remote portions not subendocardial, middle and outer fabric myocardial infarction, changes are become more apparent in the chronic phase, and appear to be the result of remote subendocardial lesions and refer to the deterioration of cardiac function[14].

Anti-inflammatory substances without deleterious effects on cardiac remodeling could interfere in heart failure evolution by create a slow down process of cardiac remodeling and improve the patient prognosis after myocardial infarction. Therefore, our primary aim was to determine the effects of borage oil in geometric myocardial remodeling on heart after myocardial infarct.

Methods

Biological model

This study was conducted in accordance with standards established by the Brazilian College of Animal Experimentation with the approval of the Ethics Committee, Universidade da Cidade de São Paulo (13398721/13392896).

Male Wistar rats, three months old, weighing 300-350 grams, were submitted to myocardial infarction by left coronary artery ligation and randomized into three groups:

- MI (n = 8) - myocardial infarcted rats without treatment.

- BO-18 (n = 10) - myocardial infarcted rats treated with Borage oil (18 mg/kg, twice a day) for one week. This dose is used in clinical practice for the treatment of symptoms of premenstrual tension and itching of atopic eczema.

- BO-180 (n = 9) - myocardial infarcted rats treated with a dose Borage oil (180 mg/kg, twice a day) for one week. This dose was selected to ensure the gamma-linolenic acid maximum conversion.

Borage oil treatment was performed immediately after complete recovery of myocardial infarction. Rats received Borage oil via gavage daily, twice a day, always in same hour (9:00 a.m. and 5:00 p.m.). This segment was considered as sub-acute and represents an appropriate phase to assessment of inflammatory response and fibrosis.

Myocardial infarction was induced by ligature of left coronary artery as described previously by Selye *et al.*[15]. Briefly,

rats were anesthetized (70 mg/kg of ketamine chloride and 11 mg/kg xylazine chloride, ip), intubated and submitted to assisted ventilation (Insight, Brazil; volume 8mL/Kg; frequency 60 cpm). After left thoracotomy, left coronary artery was identified and artery ligation was performed with nylon 6.0. The chest wall was sutured and the air within the pleural space drained. When necessary, animals received lidocaine (2 mg / kg, iv) for the treatment of ventricular arrhythmias.

Hemodynamic evaluation

Hemodynamic measurements were determined at the end of the seven-days follow-up. Animals were anaesthetized with intraperitoneal Ketamine, 50 mg/kg, and Pentobarbital, 25 mg/kg. Systemic and left ventricular blood pressures were determined via carotid catheter introduced into the thoracic aorta and into the left ventricular cavity, respectively. Hemodynamics were continuously recorded during 10 min and the average of each beat-to-beat measurement was considered for analysis.

The following parameters were computed: mean arterial blood pressure (mmHg), left ventricular end-diastolic pressure (mmHg), left ventricular systolic pressure (mmHg), maximum rate of increase of left ventricular pressure (mmHg/s), and maximum rate of decrease of left ventricular pressure (mmHg/s). The last two were used to estimate left ventricular systolic and diastolic function, respectively.

Morphometry heart

After euthanasia with overdose anaesthesia and cadmium chloride administration, the heart was removed, the atria were trimmed away, and the ventricular weight index (mg/g) was calculated by normalizing ventricular weight to body weight. Also the lungs were weighed and normalized by body weight to calculated Pulmonary Index (mg/g). The presence of congestive heart failure was confirmed when pulmonary index was higher or equal to the pulmonary index of a reference group plus 2 times the standard deviation[16]. In the present study, animals without infarct were used as the reference group (n = 7)

Ventricles were fixed by 10% formalin in a phosphate-buffered saline solution and cut into a 1–2 mm transverse slice at the equatorial plane, embedded in paraffin and cut into 5 μm sections.

Tissue sections were stained with haematoxylin–eosin (HE) and Pircro-Sirius red to morphometric measurements using a digital image analysis system (Leica Imaging Systems Ltd., Cambridge, UK)

The ratio between endocardial infarct surface length and total left ventricular endocardial circumference and the ratio between epicardial infarct surface length and total left ventricular epicardial circumference were averaged to calculate infarct size[17].

Left ventricular expansion index was calculated according to the following formula:

Left ventricular expansion index = (left ventricular cavity area/left ventricular total area) x (interventricular septum thickness/infarct wall thickness)[14]

The same formula was used to calculate left ventricular expansion index in sham animals, replacing the infarct wall thickness by the left ventricular free wall thickness.

Morphometry in the remote myocardium infarct was performed separately in two distinct regions: subendocardial and non-subendocardial. For morphometric analysis, subendocardial region was considered as a whole, the wall infarct and remote region of non-subendocardial were examined in 20 fields per histologic.

To estimate cardiomyocyte hypertrophy, HE-stained sections were examined under ×1000 magnification. The myocyte diameter (μm) around oval and central nuclei of longitudinally displayed myocytes was measured.

Leucocyte cell infiltration (cells/mm^2) was estimated in HE-stained sections under ×1000 magnification. Inflammatory cells were identified by nuclear and cytoplasmic morphological aspects. Neutrophils, lymphocytes and macrophages were identified morphologically by their appearance nuclear and cytoplasmic and were counted. Cells with morphological characteristics of fibroblasts, cardiomyocytes, endothelial vascular cells and smooth muscle vascular cells were excluded. Differential leukocyte infiltration was estimated by the ratio between cell number and area (cells / mm^2).

Collagen volume fraction (%) was estimated in Sirius red-stained sections under ×580 magnification. Collagen volume fraction was determined as the percentage of red-stained connective tissue areas per total myocardial area, excluding perivascular areas.

Statistical analysis

Data are presented as mean ± standard deviation or median (interquartile range) when appropriate.

Statistical analysis was performed using the program SigmaStat for Windows. The groups comparison was made by

analysis of variance one-way (ANOVA) or Kruskal-Wallis rank sum test, complemented by Holm-Sidak, Student-Newman Keuls test or Dunn's. The paired *t*-test was performed for comparison between non-subendocardial and subendocardial region remote to the infarct.

Simple linear regression was performed to associate the index infarct expansion with inflammatory infiltrate total differential inflammatory infiltrate and fibrosis of the non-subendocardial and subendocardial regions remote to infarct, as well as to relate fibrosis with inflammatory infiltrate total inflammatory infiltrate differential regions of the non-subendocardial and subendocardial remote to infarct. The level of significance was set at $p<0.05$.

Results

The infarct size was similar among groups (34 ± 12%, p = 0.33).

The Borage oil treatment in both dosages compared to MI group showed: lower cardiac index, lower ventricular dilation and lower myocyte hypertrophy (Table 1). Additionally, myocardial infarction without treatment group showed higher lung index which was lower in treated groups (Figure 1). These data indicate that Borage oil treatment was effective to congestive heart failure signs prevention.

TABLE 1 - Morphometric analysis of heart and lungs weight. For morphometric analysis were evaluated: heart weight, ventricular dilatation and hypertrophy of the myocytes. Treated groups had lower heart weight and lower weight lungs left ventricular dilation and reduced myocyte diameter, compared to the MI group.

Parameters	MI (n=8)	BO-18 (n=10)	BO-180 (n=9)	p
Cardiac Index (mg/g)	4.0±0.28	3.4±0.38*	3.2±0.35*	<0.001
Pulmonary Index (mg/g)	7.73±2.19	5.84±0.79*	5.59±0.78*	0.007
Ventricular Dilatation	1.0(0.70-1.78)	0.4(0.28-0.55)*	0.3(0.22-0.44)*	<0.001
Myocyte Hypertrophy (μm)	11.49±1.43	10.46±0.55*	10.33±0.70*	0.004

MI, group of animals with myocardial infarction; BO-18, group of animals with myocardial infarction and treated with 18mg/kg/day of Borage oil; BO-180, group of animals with myocardial infarction and treated with 180mg/kg/day of Borage oil. Statistical analysis was performed an analysis of variance (ANOVA), complemented by Holm Sidak test for heart weight, lung weight and myocyte hypertrophy, Dunn's Test for ventricular dilation. *p<0.05 *vs* MI. Data are expressed as mean±standard deviation or median (interquartile range).

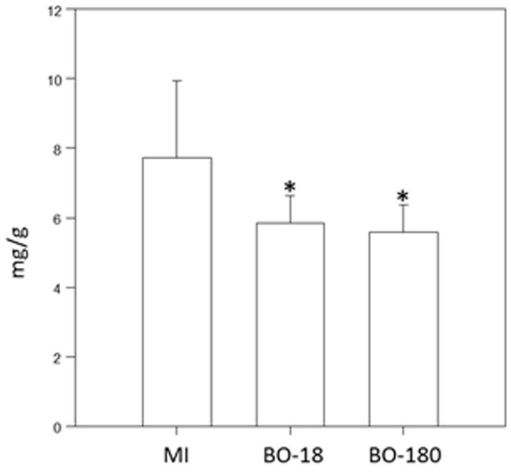

FIGURE 1 – Pulmonary index. Note that the groups treated with borage oil (BO-18 and BO-180), compared to the control group (MI) showed a significant reduction in lung index. Statistical analysis was performed an analysis of variance (ANOVA), complemented by the Holm-Sidak test. *, p = 0.007 *vs* MI. Data are expressed as mean±standard deviation.

The whole inflammatory infiltration in left ventricular infarcted myocardium was similar among groups (p=0.931). However, macrophage infiltration in left ventricular infarcted wall was increased in both treated groups compared to MI (BO-18: 61.6 (55.9-65.7); BO-180: 65.7 (54.6-75.2); MI: 38.6 (34.5-46.0), p=<0.001).

In remote to myocardial infarction regions of the non-subendocardial and the subendocardial, the whole inflammatory infiltration was mitigated by treatment of Borage oil, and more manifest in the subendocardial compared to non-subendocardial (Figure 2).

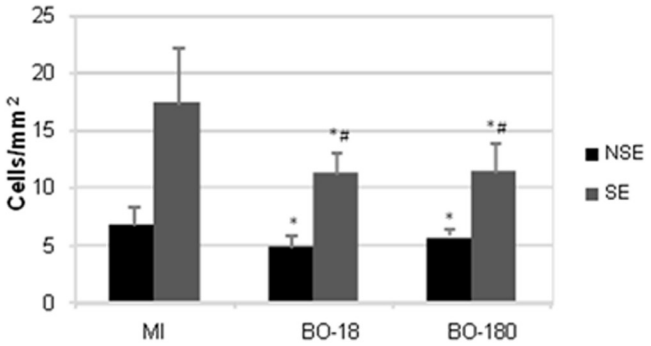

FIGURE 2 - Total inflammatory infiltrate in distinct regions of remote to infarction myocardium. Note that the groups treated with borage oil (BO-18 and BO-180) compared with control group (MI), showed a significant reduction of the inflammatory infiltrate in the area of non-subendocardium (NSE) and subendocardium (SE). Despite of this reduction, the inflammatory infiltrate was remained more intense in the SE region. Statistical analysis was performed an analysis of variance (ANOVA), complemented by the Holm- Sidak test. The Student t test was used for intragroup comparisons (NSExSE). *p<0.05 *vs* MI. #p<0.05 *vs* NSE. Data are expressed as mean ± standard deviation.

The neutrophil infiltration in the non-subendocardial, subendocardial and infarct were comparable among groups (p=0.862, p=0.547 and p=0.375, respectively).

The lymphocyte infiltration was reduced in remote regions and macrophage infiltration was reduced in the remote subendocardial in both treated groups compared to the MI group. There was a higher proportion of macrophages to lymphocytes in this region (Figure 3).

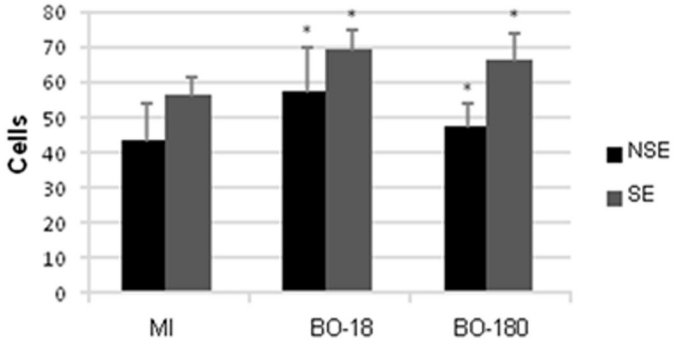

FIGURE 3 – Relationship Macrophages/Lymphocytes. After differential analysis of the inflammatory infiltrate in the remote regions of the myocardial infarction, non-subendocardium (NSE) and subendocardium (SE), we observed there was an increase of macrophage-lymphocyte ratio in the two regions studied (NSE and SE), in the treated groups (BO-18 and BO-180). Statistical analysis was using the Student-Newman-Keuls Test *p <0.05 *vs* MI. Data are expressed as mean ± standard deviation.

The fibrosis of infarcted myocardium was comparable among groups (p=0.944). In the remote region of non-subendocardial and subendocardial, myocardial fibrosis was lower in treated groups compared to MI (Figure 4).

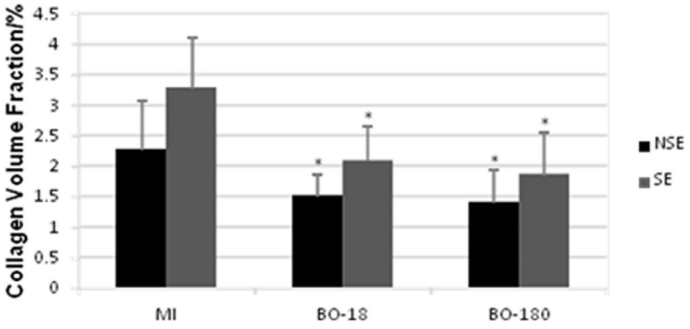

FIGURE 4 – Fibrosis in the remote region of infarction. The fibrosis, estimated by the collagen volume fraction in remote regions to MI. It notes that there was a reduction in fibrotic in region non-subendocardial (NSE) and subendocardial (SE) in both treated groups (BO-18 and BO-180). The statistical analysis was used analysis of variance (ANOVA), complemented by the Holm Sidak test. *p<0.05 *vs* MI. Data are expressed as mean ± standard deviation.

There was a positive correlation of ventricular dilation and the whole inflammatory infiltration, lymphocytes and macrophages infiltration in the remote subendocardial region (Figure 5). Considering remote non-subendocardial region features, ventricular dilatation was positively correlated to lymphocytes infiltration (Figure 6).

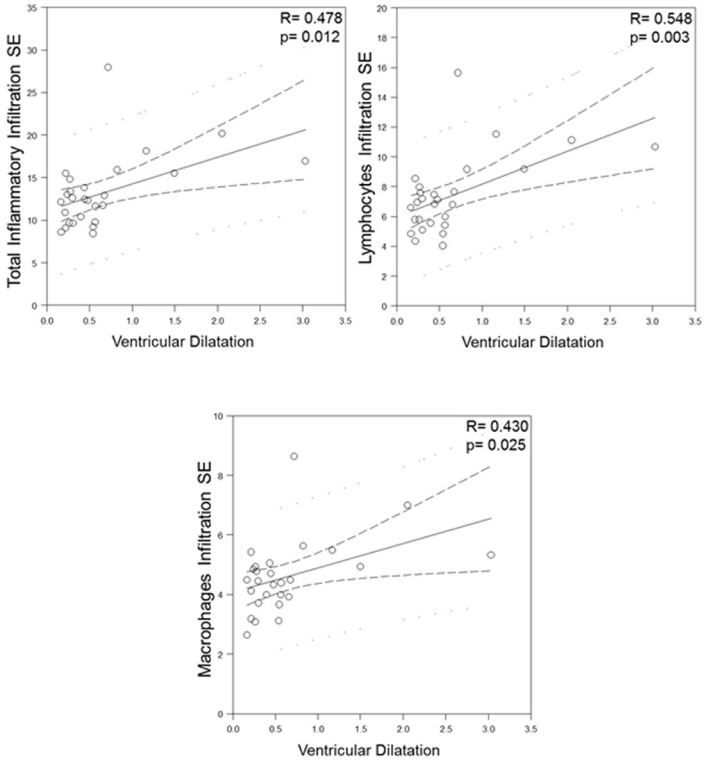

FIGURE 5 - Positive correlation between the total inflammatory infiltration, inflammatory infiltration of lymphocytes and macrophages of the remote region to myocardial subendocardium (SE) with ventricular dilation.

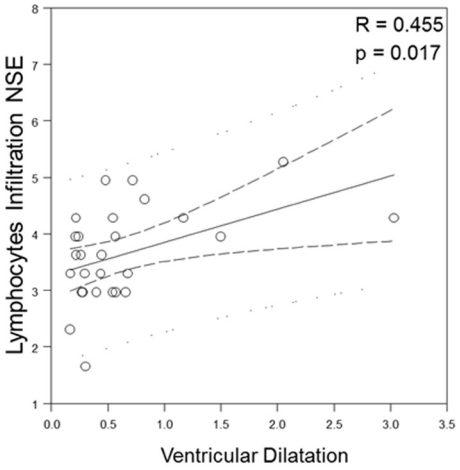

FIGURE 6 - Positive correlation between the inflammatory infiltrate of lymphocytes of the remote region to myocardial non-subendocardium (NSE) with ventricular dilation.

All data of hemodynamic was similar among groups ($p>0.05$ and data not described).

There was a negative correlation between fibrosis and macrophages/lymphocytes ratio in the remote subendocardial region (Figure 7).

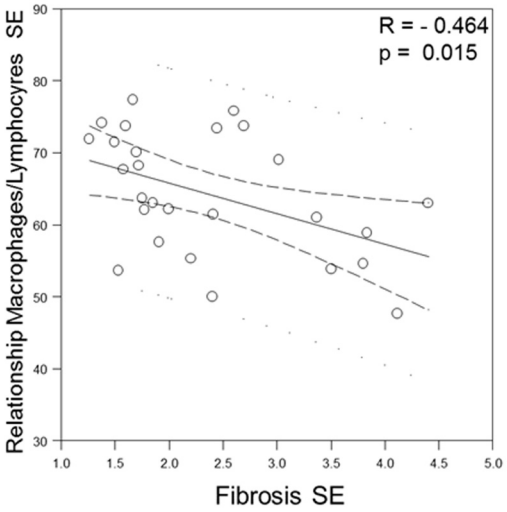

FIGURE 7 - Negative relationship between fibrosis and macrophages/lymphocytes in the remote region to myocardial subendocardium (SE).

Discussion

The present study showed modulation of progression of cardiac remodeling after myocardial infarction with Borage oil treatment in rats. The inflammatory response attenuation and subsequent reduced fibrosis deposition in viable myocardium remote to infarction prevented ventricular dilatation and heart failure.

Changes of tension and stress in the left ventricle after myocardial stimulate myocyte hypertrophy. The borage oil treatment promoted a low diameter of myocytes, low cardiac index and low pulmonary index, indicating prevention of congestive heart failure signs in both doses used. Despite of two different doses of Borage oil, we found similar responses, indicating that 18 mg/kg twice a day - dose recommended to treatment of atopic eczema or premenstrual tension in humans - is appropriate for treatment, and this can be explained by the limited conversion of gamma-linolenic acid in dihomo-gamma-linolenic acid, via alongase activity. This conversion in dihomo-gamma-linolenic acid to arachidonic acid, via delta-5 desaturase activity, is auto-limited[18].

Inflammatory infiltration response

In our study, Borage oil attenuated the lymphocytes and macrophages response in regions remote to the infarct. Current findings point toward the hypothesis that the Borage oil treatment increased the bioavailability of dihomo-gamma-linolenic acid which may have acted directly or via increased formation of anti-inflammatory eicosanoids via cyclooxygenase activity[7-9]. The omega-6 showed anti-inflammatory effects in treating patients with rheumatoid arthritis, atopic dermatitis, psoriasis syndrome and premenstrual[4,5].

Dihomo-gamma-linolenic acid can modulate the inflammatory response via T-lymphocytes and monocytes, while reducing secretion of tumor necrosis factor and interleukin 1 and 2, independently of prostaglandin formation[9,19]. The findings of this study suggest that Borage oil can be an alternative to anti-inflammatory treatment after myocardial infarction, preventing post-infarction aneurysm formation, rupture and ventricular dilatation.

Reduction of macrophage infiltration may have occurred because of dihomo-gamma-linolenic acid and direct action in response to reduced release of pro-inflammatory cytokines. The use of steroidal or non-steroidal anti-inflammatory drugs in rats showed infarcted aneurysm formation in repair scar thinning, increased dilation and increased mortality[12].

Treatment with Borage oil prostaglandin increases the ratio E1/E2 by raising the availability of prostaglandin E1 via dihomo-gamma-linolenic acid metabolism, and its can be modulate beneficially inflammatory response due to prostaglandin with anti-thrombotic and anti-proliferative properties[7,19]. The administration of prostaglandin E1 despite showing good results becomes inconvenient because of its short half-life[20]. Borage oil can be an alternative way of offering prostaglandin E1 for the treatment of different inflammatory diseases.

Despite if increased macrophage infiltration, myocardial infarction region developed appropriated scar formation, preventing aneurysm formation, occurrence of disruptions, and dilation of cardiac chamber by expansion of the infarcted wall and subsequent congestive heart failure.

Inflammation and fibrosis relationship

After myocardial infarction, remote subendocardial lesions are associated with ventricular dilation, as previously demonstrated, indicating its importance to cardiac remodeling[14]. In our results, it was observed attenuation of total inflammatory infiltration in remote subendocardial and non-subendocardial regions. The remote subendocardium was the region most susceptible to inflammation, as previously demonstrated by our

studies[14,17]. In present study, the attenuation of the inflammatory response was most evident in remote subendocardium after Borage oil treatment. It is believed that more significant changes in the remote non-subendocardium can be found later as a result of remodeling that occurs in the fibrosis[14].

Several drugs and anti-inflammatory corticosteroids and inhibitors of angiotensin-converting enzyme were used to attenuate remodeling and fibrosis in remote myocardium after myocardial infarction. However, these treatments had deleterious effects on the healing process of the infarcted area, as scar with deformations and changes in the amount of collagen[12,13]. In our study, treatment with Borage oil reduced fibrosis in remote myocardium regions without altering fibrosis in the infarcted region.

Fibrosis can be modulated by inflammation. Different cell types have different answers. For example, macrophages can be classified into M1 and M2. The M1-type macrophages are related to the pro-inflammatory state, while the M2 type macrophages are related to the state with anti-inflammatory and tissue repair. Several studies suggest that the macrophages have high plasticity and are able to adapt to different situations[21-23]. Current data shows that the macrophage infiltration in the wall region of infarct presented profile for synthesis and release of growth factors and transformation, which stimulate myofibroblasts differentiation and promote collagen synthesis.

Limitations

The effects of Borage oil treatment were observed by histomorphology alterations in cardiac tissue in the acute phase after myocardial infarct. There are some limitations: a) the absence of assessment of pro- and anti-inflammatory cytokines molecular expression, metalloproteinases activities and growth and differentiation factors expression; b) fibroplasia and bioavailability of fatty acids after administration of the doses were not evaluated; c) borage oil treatment after IM was aimed to assess the effects of treatment in inflammation and fibrosis. Myocardial inflammatory infiltration, fibrosis and ventricular expansion were improved by Borage oil treatment. However, our data not shown improvement in hemodynamic or cardiac function with treatment. One hypothesis is that the phase chosen for analysis, seven days after MI, represents a critical phase for cardiac tissue remodeling but still early to hemodynamic and functional effects.

Preventive Borage oil treatment or studies conducted in chronic phases of myocardial infarction could be more adequate to demonstrate functional changes related to cardiac remodeling.

Clinical implications

Clinical studies indicate that circulating levels of omega-6 and omega-3 are smaller in Italian patients with recent MI[24], as well as the circulating levels of linoleic acid can relate to lower total mortality and cardiovascular disease in elderly patients[25]. In these studies, it was observed that the dose-response relationship and potential of omega-6 are still controversial, difficult standardization ideal consumption[26].

Borage oil introduced as diet complement in myocardial infarcted patients can become a way to modulate the inflammatory response and its consequences in the acute phase after myocardial infarction.

Conclusion

Borage oil treatment attenuated cardiac remodeling and congestive heart failure after experimental myocardial infarction.

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