Protective effect of apolipoprotein AI mimetic peptide 4F on renal and cardiac injury and endothelial dysfunction induced by acute myocardial infarction in hypercholesterolemic rats receiving iodinated contrast

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ABSTRACT

Moreira, R.S, Irigoyen, M.C, Capcha, J.M.C, Sanches, T.R, Gutierrez, P.S, Garnica, M.R, Noronha, I.L, Andrade, L. Protective effect of apolipoprotein AI mimetic peptide 4F on renal and cardiac injury and endothelial dysfunction induced by acute myocardial infarction in hypercholesterolemic rats receiving iodinated contrast.

INTRODUCTION: The use of contrast after angiography in infarcted animals induces acute kidney injury, being associated with worsening prognosis and increased mortality. Hypercholesterolemia is an aggravating factor of endothelial injury in acute myocardial infarction (AMI) and the use of contrast in diagnosis and treatment can cause acute kidney injury. Treatment with the apolipoprotein AI mimetic peptide 4F can reverse endothelial injury by reducing LDL levels and preventing its oxidation. OBJECTIVES: To analyze the effect of Apo A-I (using mimetic peptide 4F) on cardiac and renal injury induced by acute myocardial infarction (AMI) with contrast therapy in hypercholesterolemic rats. METHODS: This was a prospective study with rats on a diet at 4% cholesterol for 8 days, divided into a SHAM group operated without coronary ligation (n=6) or infarcted animals with ligation of the left anterior descending coronary artery, with or without the use of contrast and treatment 6 hours after infarction induction: AMI (n=15), AMI+C (iopamidol 2.9 g/kg body weight, intrafemoral artery injection n=15), AMI+4F (4F, 10mg/kg body weight, peritoneal injection, n=8) and AMI+C+4F (n=8). All results are analyzed after 24 AMI and expressed as mean and standard error. RESULTS: It was observed that the AMI+4F and AMI+C+4F groups showed a better response to cardiac injury and renal injury compared to the AMI and AMI+C groups. There was an improvement in renal function through 12-hour creatinine clearance, increased expression of eNOS, increased VEGF, preservation of mitochondrial morphology, reduction of inflammation with a lower expression of CD68+ (macrophages), decreased positive tunnel cells associated with an increase in apolipoprotein AI (Apo AI) expression in renal tissue. The same happened in cardiac function with decreased plasma troponin, increased expression of eNOS, VEGF, isolectin B4, reduction in inflammation represented by lower TLR4 expression, positive tunnel cells,

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improved cholesterol profile, preservation of mitochondrial morphology and associated with increased expression of Apo AI in cardiac tissue. Hemodynamics were preserved with improvement in cardiac output, ejection fraction, baroreflex response, left ventricular end-diastolic pressure, and associated with a decrease in the infarct area measured by both echocardiogram and immunohistochemistry. We demonstrate that treatment with apolipoprotein AI can be a therapeutic option in cardiac and renal injury by reversing the inflammatory response through the efflux of HDL-dependent cholesterol.

Keywords: Apolipoprotein, Hypercholesterolemia, Contrast, Acute myocardial infarction/surgery, Acute kidney injury, Contrast nephropathy.

INTRODUCTION

MECHANISMS OF HYPERCHOLESTEROLEMIA;

The effects of hypercholesterolemia act on the proliferation and differentiation of hematopoietic stem cells/progenitor cells (CEPH). In addition, dyslipidemia also acts on neighboring cells of the CEPH and causes inflammation, arteriosclerosis and cardiovascular diseases. [1, 2, 3]

Hypercholesterolemia is associated with phenotypic changes in endothelial cell function that lead to a pro-inflammatory and pro-thrombotic state in different segments of the microcirculation. [4, 5]

Atherogenesis follows three phases, being endothelial dysfunction, fatty plaque formation, and fibrous capsule development. The most discussed hypothesis for its etiology is that the initial lesion occurs in response to an alteration of the endothelium caused by disturbances in blood flow (shear stress), the presence of oxidized lipids in lipoproteins, or the presence of some infectious agent. [6]

EFFECTS OF ATHEROSCLEROSIS IN AN ANIMAL MODEL;

Systemic sclerosis related to a diet high in cholesterol causes an autoimmune disease of the connective tissue characterized by decreased vascular function, increased oxidative stress, impaired angiogenesis and inflammation in the internal organs developing crosstalk mainly related to kidney function.[7, 8]

Studies using a myocardial infarction model in hypercholesterolemic rats have shown an increase in LDL compared to HDL, mimicking the situation found in humans.[9] Animals fed a high-cholesterol diet showed a worsening of ventricular remodeling compared to animals fed a normal diet. This study demonstrates that the hypercholesterolemic diet can impair heart function leading to a decrease in effective cardiac output and harming other organs. [9, 10]

High cholesterol in a rat model of hropercholesterolemia showed an increased myocardial vulnerability with worsening of the infarct area, significant alteration in the number of apoptotic cells and associated with the activation of endoplasmic reticulum stress pathways.[11, 12]

ALTERATIONS OF ACUTE MYOCARDIAL INFARCTION;

In the presence of an acute myocardial infarction, there is a process of ischemia of the myocardial muscle, which can lead to necrosis of part of the musculature due to lack of adequate supply of oxygen and nutrients, leading to apoptosis of the heart cells.[13]

Myocardial infarction is one of the main causative agents of heart failure, with a high rate of morbidity and mortality, leading to a high cost treatment in public hospitals, and with the increase in the perspective of age, this cost tends to increase. Despite therapeutic advances in treatment, the

prognosis is still poor, which can lead to death in a short period of time or heart failure. Studies with new therapeutic interventions are essential for advances in the treatment of the disease.[14]

The choice of a model of acute myocardial infarction in animals that determines physiological changes found in clinical cases with humans is extremely important for understanding cardiovascular diseases. In the same AMI model we used, baroreflex sensitization, reduced cardiac parasympathetic modulation, and increased cardiovascular sympathetic modulation were confirmed.[15]

Echocardiogram examination is a reliable and commonly used method to examine cardiovascular diseases. This modern technology offers a new opportunity to study cardiac dysfunction after acute myocardial infarction (AMI) in rats. Cardiovascular function parameters can provide a detailed prognosis of ejection fraction, cardiac output and especially infarct area in animals.[16, 17]

Troponin I (cTnI) plays an important role in the evaluation of the ischemic area and prognosis of the patient. In an animal model, the peak of troponin I alteration was demonstrated 24 hours after infarction induction and the correlation of infarct expansion area with worsening of this marker in rats. It has been identified as an excellent marker of myocardial injury in rats associated with the prognosis of treatment.[18, 19]

In infarction, the activation of leukocytes induces the release of pro-inflammatory cytokines, which in the acute phase cause alteration of the immune system and propagation of the systemic inflammatory response, leading to intravascular and hemodynamic dysfunction.[20]

In a rat model of AMI with ligation of the anterior descending coronary artery of the left ventricle, after twenty-four hours the animals showed a significant decrease in ejection fraction associated with the increase in infarct area and reproducing what occurs in humans.[21]

Acute myocardial infarction associated with hypercholesterolemia in an animal model showed a significant worsening in the untreated group with an increase in LDL, triglycerides and decreased HDL in plasma. A significant reduction in the expression of e-NOS and VEGF, and an increase in the infarct area with apoptosis in the cardiac tissue of rats. [22]

Myocardial ischemia alone in an animal model has already led to significant alteration in cardiac tissue, demonstrating an increase in TLR4 (Toll-like receptor 4), which is an important inducer of apoptosis, demonstrated through the P38 pathways, MAPK, Bax, Bcl-2, cleaved caspase-3 and c-Jun/AP-1.[23]

THE RISKS OF USING LOW MOLECULAR WEIGHT CONTRAST

Despite the measures to protect renal function in the examination of catheterizations or angioplasty, the use of low molecular weight contrast in acute myocardial infarction (AMI) is related

to a worsening of renal function in high-risk patients who have several underlying diseases associated with dyslipidemia and is related to a high risk of mortality.[24]

Contrast-induced nephrotoxicity (CIN) has been discussed as one of the causes of acute kidney injury (AKI) in in-hospital patients. In patients with risk factors, the incidence is much higher, with a 25% increase in baseline creatinine in a period of 48 to 72 hours after the use of contrast. Decreased glomerular filtration rate (GFR) may lead to the use of hemodialysis therapies that are invasive and contribute to worsening prognosis.[25]

Heart failure with reduced effective arterial volume may lead to a higher risk of AKI in the use of low molecular weight contrast, especially in the use of vasoactive drugs that potentiate the action of nephrotoxicity.[26]

In an experimental study with hypercholesterolemic rats, animals that received low molecular weight contrast showed a worsening of renal function compared to animals that did not.[27] In a model of nephrectomy of the right kidney and ischemia with renal reperfusion in rats, hypercholesterolemic animals showed a decrease in HDL associated with an increase in LDL, and when submitted to renal ischemia, they presented a worsening of renal function measured by inulin clearance.[28]

Using a model of contrast-induced nephropathy in rats, they administered a dose of 2.9 g/kg of iopamidol and demonstrated a worsening of renal function measured by the increase in tubular necrosis.[29] In a model of contrast-induced kidney injury, rats showed a worsening of renal function as measured by creatinine clearance, which was maintained even after six days of use and is related to apoptosis of tubular cells in the kidney.[30]

In another study of contrast-induced nephropathy with the use of a dose of 2.9 g/kg of iopamidol, rats showed apoptosis of epithelial cells in renal tubules by activation of the JnK (Jun N-terminal kinase) and P38 (mitogen-activated protein kinase) pathways, and the use of contrast was related to the inflammatory process, apoptosis and renal injury.[30, 31]

CROSSTALK BETWEEN HEART AND KIDNEY IN THE USE OF CONTRAST

Crosstalk is a cross-activation between a primary lesion and dysfunctions to the other organs. In patients with low cardiac output, there was a significant increase in plasma creatinine on angiography with contrast.[32]

Decreased effective renal artery blood flow and vasoconstriction are considered major mechanisms of acute kidney injury. The use of contrast in addition to low cardiac output after suffering an acute myocardial infarction is associated with a worsening of renal function.[32, 33]

However, hypercholesterolemia is also associated with worsening renal function, and studies show that an increase in creatinine during acute myocardial infarction increases the risk of mortality

in patients independent of other conventional risk factors. Not to mention the use of contrast, which can further aggravate the patient's condition at a time of hemodynamic instability.[34]

Increased plasma creatine is associated with increased infarct area in patients who have experienced acute myocardial infarction.[35]

Acute myocardial infarction leads to a chronic heart disease causing heart failure, acute kidney injury can cause a long-term chronic kidney disease and considering this scenario cardiorenal syndrome and a possibility of worsening in patients with crosstalk.[36, 37]

Patients who have increased risk factors during the invasive procedure with the use of contrast in diagnostic or interventional examination may develop acute kidney injury when not properly treated.[38]

HEMODYNAMIC STUDY OF HEART RATE (HR), ARTERIAL PRESSURE (BP) AND BARORECEPTORS IN MYOCARDIAL INFARCTION

Hemodynamic control in myocardial infarction is important to reduce ischemic complications and avoid myocardial overload. Blood pressure at normal levels is critical for maintaining tissue and organ perfusion.[39]

Impaired baroreflex function is a factor responsible for poor prognosis in patients with acute myocardial infarction. In a time of cardiac stress, the maintenance of cardiovascular homeostasis is dependent on the action of arterial pressoreceptors, cardiopulmonary receptors that reduce cardiovascular distress.[40]

Either the sympathetic system that controls the vessels and heart through efferent fibers, or the parasympathetic system using the vagus nerve to the heart. These commands, controlled by the aortic and carotid pressoreceptors, are responsible for the efficient maintenance of cardiac output and peripheral resistance.[41]

Pressoreceptors can increase parasympathetic activity by acting on the regulation of heart rate and blood pressure. The regulation of cardiac vagal tone is modulated by pressoreceptors, which is responsible for resting vagal tone in the animal when awake and using breathing voluntarily and always remembering that chemoreceptors and other reflexes associated with the animal's breathing can alter the activity of the parasympathetic system.[42]

Cardiovascular diseases, in a general context, present alterations in sympathetic activity that are better known and studied. However, there is a consensus on the parasympathetic system, relating preserved vagal function to adequate maintenance of blood pressure variability and better protection of target organs.[43]

Heart rate variability can determine a patient's prognosis, when it is reduced it increases the risk of sudden death during acute myocardial infarction.[44]

HIGH-DENSITY LIPOPROTEIN (HDL);

The control of HDL levels has been useful for assessing cardiovascular risk and studies have shown the correlation between inflammation and HDL dysfunction. Functional measurements of HDL levels contribute important information about the activity of the molecule and are involved in atherogenesis, inflammation, and infection.[45, 46]

The anti-atherosclerotic action is associated with several mechanisms of protection of the HDL molecule, as demonstrated in a study in the European population: high HDL levels decrease the risk of cardiovascular diseases even in type 2 diabetic patients. [47]

Cellular cholesterol efflux plays an important role in reverse cholesterol transport (CRT), which occurs in active or passive diffusion mechanisms. Among the active processes are those involving the interaction of HDL, where mature HDL2 and HDL3 molecules are generated from the free lipids of apo AI or lipids poor in pre-beta HDL. These precursors are produced as nascent HDL from the liver, intestine or are released from the lipolyzed VLDL molecule and chylomicrons.[48]

Cholesterol is actively pumped out of cells by the ATP-binding cassette transporter A1 (ABCA1), mediated by lipid efflux and phospholipid transfer protein (PLTP) in lipid-poor apo AI. Lecithin-cholesterol acyltransferase (LCAT), which esterifies cholesterol in HDL, plays an important role in CRT because cholesteryl esters are much more hydrophobic than cholesterol and become trapped in the nuclei of cholesterol molecules.

A portion of the free lipid of apo A-I undergoes glomerular filtration in the kidneys. [48, 49]

PROTECTIVE ACTION OF LIPOPROTEINS;

High-density lipoproteins (HDL) are involved in cholesterol efflux. The mechanism of action of HDL is still not well understood, due to the lack of information about the structure of the molecule. In its composition we find apolipoproteins that are part of the HDL molecule and its fractions, and are contained in lipoprotein particles. They play important roles in lipoprotein metabolism, such as transport of these hydrophobic molecules in the plasma aqueous medium, binding to specific receptors on the cell surface, and activation or inhibition of enzymes involved in lipid metabolism.[50, 51]

Apolipoprotein A-I (apo A-I) is the largest component of the high-density lipoprotein (HDL) particle, contains about 190-243 amino acids in its composition, acts as a cofactor for the enzyme lecithin cholesterol acetyltransferase, and plays a key role in lipid binding and HDL molecule formation. It also acts as a mediator in the transfer of cholesterol from cells to HDL particles and its plasma concentration of apo A-I is strongly associated with HDL.[52-54]

THE USE OF APOLIPOPROTEIN AI MIMETIC PEPTIDE 4F;

The search for peptides smaller than apo A-I, but with the same lipid-binding properties, led to the synthesis of a peptide with 18 amino acids. Its sequence (D-W-L-K-A-F-Y-D-K-V-A-E-K-L-K-E-A-F) is not identical to the original structure of apo A-I, however it has a formation of amphipathic α-helices similar to those found in apo A-I and mimics many of the lipids of apo A-I.[55]

4F is the main mimetic peptide of Apo A-1. It has been shown to play an important role in inflammatory states.[56]

The 4F mimetic peptide (4F) features 4 strongly hydrophobic phenylalanine residues that allowed greater penetration of water molecules into the hydrophobic environment. The penetration of the 4F mimetic peptide occurs through phosphatidylcholine, a phospholipid present in cell membranes and demonstrated through in vitro studies that proved the presence of 4F inside cells.[55, 57]

Plasma HDL concentrations decrease with age in prospective studies. Decreased HDL concentration and function may occur secondary to hormonal changes, inflammatory processes, and diabetes mellitus. In addition to these specific effects, the aging process may be involved with a decrease in the concentration of HDL and its functions. HDL deficiency is extremely rare among centenarians. HDL can modulate the aging process, not only because of its well-known antiatherogenic function, but possibly also because it directly interferes with aging by signaling proteins such as klotho. Most of the current results, however, are based on cell culture and experiments with transgenic animals. There are no studies on models *in vivo*.[58]

The anti-inflammatory and anti-atherogenic effects of 4F are due to the elevation of HDL formation, increased cholesterol efflux and reduced lipoprotein oxidation. In addition, improvement of arterial vaso-reactivity is also an important function of the .4F[46] Currently, plasma levels of apolipoproteins A-I have been described as better predictors of atherosclerotic diseases. Oral administration of apo A-I 4F dramatically inhibits atherosclerosis in mice independent of changes in plasma HDL and total cholesterol levels.[59]

A recent study showed that administering apo A-I (4F) to mice resulted in an increase in HDL, a reduction in lipoprotein lipid peroxides, an increase in cholesterol efflux, a decrease in inflammatory cells, a reduction in plasma LDL levels, and activation of the paraoxonase pathway, favoring the conversion of pro-HDL molecules with anti-inflammatory action.[60]

A study with the use of apo A-I D-4F improved the healing of endothelial lesions of carotid arteries in mice in the hypercholesterolemia model. It showed a significant decrease in oxidative stress and improvement in lipid oxidation. [61]

Apo A-I 4F protected against atherosclerosis in an animal model. Mechanisms include reverse cholesterol transport, removal of low levels of oxidized lipids, and preventing LDL

oxidation. Intraperitoneal administration of apo A-I 4F enhanced HDL's ability to protect against LDL oxidation in a diet-induced atherosclerosis model in mice.[62]

There was no difference in oral or intraperitoneal administration, both showed a significant improvement in atherosclerotic lesion in an apo-null mouse model with graft from the inferior vena cava in the right carotid.[63]

In some review studies with the use of apo A-I 4F, the positive action of the synthetic peptide in the prevention and reduction of atherosclerosis in animal models was confirmed. Its positive effect has been demonstrated in rats, mice, monkeys, and rabbits.[64-66]

Even in a model of cardiac hypertrophy in mice, with prolonged use of a hypercholesterolemic diet, the protective action of apo A-I 4F was confirmed through the improvement of cardiac function, increase of HDL and decrease of LDL.[67]

This drug may show benefits especially in acute coronary syndrome and exerts a protective function in systemic inflammatory response syndrome.[68]

Experimental studies have shown proven benefits on cardiovascular function, with an increase in antioxidant and anti-inflammatory molecules, and an increase in paraoxonase and eNOS.[69-71]

Recently, I demonstrated through a study conducted with rats in the sepsis model, the intraperitoneal administration of apo A-I 4F promoted the formation of new HDL particles. This study demonstrated improved cardiac performance, kidney function, and increased survival of the mice.[66, 72]

Pharmacokinetic and pharmacodynamic studies in the oral use of apo A-I 4F were conducted, testing the safety of the peptides in humans. The results were satisfactory, with an increase in HDL and an improvement in its anti-inflammatory activity. [73]

Some pharmaceutical companies of great importance in the world market are already testing the use of apolipoprotein AI in humans, phase 1, 2 and 3 clinical trials are already underway, demonstrating the importance of using this peptide in future treatments.[74]

Currently, the study of peptide 4F is already in the preclinical phase for the treatment of coronary heart disease and atherosclerosis.[75]

The beneficial effects of HDL on atherosclerosis have been attributed to apo A-I. It can be used as a therapeutic intervention. However, as it is a very large protein, it is very difficult to produce and is also extremely expensive. Due to this problem, Apo A-I mimetic peptides have been produced that can be used in the treatment of atherosclerosis with therapeutic success of reducing atheroma plaque and improving myocardial vascularization.[74, 76]

Apolipoprotein A-I was investigated in a randomized, double-blind, multicenter study, and the pharmacokinetic and pharmacodynamic safety of the infusion in patients with stable

atherosclerotic disease was evaluated. The results showed a favorable overall safety, without the presence of hepatic and renal toxicity and with an increase in the concentration of apo A-I in the plasma, improving the cholesterol efflux.[77]

In a recent study, it was demonstrated that the use of the bromodomain and extraterminal domain (BET) inhibitor, increases the production of transcription of the Apo A-I gene in humans, showed an increase in the number of HDL molecules associated with apo A-I with effective action in the improvement of pro-inflammatory, pro-atherosclerotic and pro-thrombotic pathways that may contribute to the risk of Cardiovascular Disease.[78]

HIP

Based on the scientific evidence analyzed, we hypothesized that hypercholesterolemic, infarcted and contrast-enhanced rats could present cardiac and renal manifestations similar to those observed in hospitalized patients. It is worth noting that the previous treatment of cardiac and renal lesions is essential for the prognosis of patients.[79]

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OBJECTIVE

The main objective of this project was to analyze the effect of Apo A-I (using mimetic peptide 4F) on cardiac and renal injury induced by acute myocardial infarction (AMI) with contrast therapy in hypercholesterolemic rats.

SPECIFIC OBJECTIVES

- To interpret cardiac structural and functional parameters by transthoracic echocardiography in 8-week-old Wistar rats, infarcted hypercholesterolemic animals, as well as infarcted hypercholesterolemic animals using contrast with and without 4F apo AI treatment and comparing them to controls.
- To identify morphological alterations of the mitochondria of the heart and kidney in 8-week-old Wistar rats, infarcted hypercholesterolemic animals, as well as hypercholesterolemic infarcted animals with the use of contrast with and without 4F apo AI treatment and comparing them to controls.
- To verify whether the expression of apo AI 4F is increased in the heart and kidney of 8-week-old Wistar rats, sham animals, hypercholesterolemic infarcted animals, as well as hypercholesterolemic animals infarcted with contrast and comparing them to animals treated with apo AI 4F.
- To provide data on lipid metabolism disturbance in 8-week-old Wistar rats, infarcted hypercholesterolemic animals, as well as hypercholesterolemic infarcted animals with

contrast therapy with and without 4F apo AI treatment and comparing them to controls.

- To investigate the inflammatory response and angiogenesis mechanisms involved in the vascular system of the heart and kidney of 8-week-old Wistar rats, infarcted hypercholesterolemic animals, as well as hypercholesterolemic infarcted animals with contrast with and without 4F apo AI treatment and comparing them to controls.
- To control left ventricular end-diastolic pressure (LVEDP), vasomotor response, baroreflex in 8-week-old Wistar rats, infarcted hypercholesterolemic animals, as well as infarcted hypercholesterolemic animals with the use of contrast with and without 4F apo AI treatment and comparing them to controls.

MATERIALS AND METHOD

The experimental procedures were developed in accordance with the institutional guide for laboratory care and use, with the approval of the Ethics Committee on the Use of Animals (FMUSP – CAPPESQ) School of Medicine of the University of São Paulo, Brazil/ Ethics Committee for Analysis of Research Projects (#261/13).

The animal model used in our project consists of a strain of male Wistar rats with weights between 200 – 250g and about 8 weeks of age were obtained from the Central Vivarium of the Faculty of Medicine of the University of São Paulo (FMUSP). The animals were kept separately in an environment with controlled temperature (22 – 24ºC) and light (12-hour light/dark cycle). They had free access to water and the hypercholesterolemic diet was prepared according to the standard diet for rodents AIN-93G and modified by replacing 3.5% of soybean oil with hydrogenated fat and adding 4% of cholesterol and 0.4% of cholic acid.[80]

The animals were randomly divided into five groups listed below.

We used the AMI (acute myocardial infarction) model described by Pfeffer et al, 1979.[81]

EXPERIMENTAL SEQUENCE;

All animals received a 4% cholesterol diet and were separated into 5 groups:

3.1.1). Sham **(S);**

3.1.2). Infartado **(I);**

3.1.3). Contrast infarction **(CI);**

3.1.4). Infarctionary treated with APO AI 4F **(I+4F);**

3.1.5). Contrast-enhanced infarction treated with APO AI 4F **(IC+4F).**

The above-mentioned experimental groups were studied after five distinct periods: 8th day, 00h on the 9th day, 6h on the 9th day, 12h on the 9th day and 10th day. It is important to emphasize that infarcted animals were included in the protocol only when an area of akinesia was confirmed (by

echocardiography) and troponin examination 24 hours after the surgical procedure to induce infarction. It should also be noted that those with a minimum of 15% of akinesia area in the 24 hours were considered for the study.

Next, procedures common to all studies will be presented. It should be noted that this methodological sequence presented will be maintained in the presentation of the results and in their discussion.

Sham Group 8 Days Cholesterol 4% Diet (SHAM);

0h: Weighing, cannulation of the femoral artery and vein.

6h: The volume of the 0.9% DES vehicle was administered intravenously (IV) using a femoral artery for each animal and were equivalent to the volume of the 0.9% SF vehicle administered intraperitoneally (ip) for each animal and were equivalent to the volume of the Apo AI 4F.

12 noon: Beginning of diuresis control for 12 hours in a metabolic cage.

24h: Blood pressure, heart rate (30 min) recording, baroreflex sensitivity assessment, echocardiographic evaluation and left ventricular end-diastolic pressure measurement, and euthanasia to remove plasma for biochemical analysis and tissue for molecular biology analysis.

Group 8 days of diet 4% Infarcted Cholesterol (AMI);

0h: Weighing, artery and vein cannulation and AMI

6h: The volume of the 0.9% DES vehicle was administered intravenously (IV) using a femoral artery for each animal and were equivalent to the volume of the 0.9% SF vehicle administered intraperitoneally (ip) for each animal and were equivalent to the volume of the Apo AI 4F.

12 noon: Beginning of diuresis control for 12 hours in a metabolic cage.

24h: Blood pressure, heart rate (30 min) recording, baroreflex sensitivity assessment, echocardiographic evaluation and left ventricular end-diastolic pressure measurement, and euthanasia to remove plasma for biochemical analysis and tissue for molecular biology analysis.

Group 8 days of diet 4% Contrast Infarction (AMI+C);

0h: Weighing, artery and vein cannulation and AMI

6 a.m.: Injection of iopamidol contrast 2.9 g iodine/kg body weight in the femoral artery and volume of the vehicle with 0.9% DES was administered by ip, for each animal, and were equivalent to the volume of Apo AI 4F.

12 noon: Beginning of diuresis control for 12 hours in a metabolic cage.

24h: Blood pressure, heart rate (30 min) recording, baroreflex sensitivity assessment, echocardiographic evaluation and left ventricular end-diastolic pressure measurement, and euthanasia to remove plasma for biochemical analysis and tissue for molecular biology analysis.

Group 8 days of 4% cholesterol diet Infarction, treated with APO AI 4F (AMI+4F);

0h: Weighing, artery and vein cannulation and AMI

6 a.m.: The volume of the 0.9% DES vehicle was administered intravenously, using a femoral artery, to each animal and were equivalent to the contrast volume. They received treatment with APO AI IP/10mg/kg.

12 noon: Beginning of diuresis control for 12 hours in a metabolic cage.

24h: Blood pressure, heart rate (30 min) recording, baroreflex sensitivity assessment, echocardiographic evaluation and left ventricular end-diastolic pressure measurement, and euthanasia to remove plasma for biochemical analysis and tissue for molecular biology analysis.

Group 8 days of diet cholesterol 4% Contrast Infarction Treated with APO AI 4F (AMI+C+4F);

0h: Weighing, artery and vein cannulation and AMI

6h: Injection of iopamidol contrast 2.9 g iodine/kg body weight in the femoral artery and treatment with APO AI IP/10mg/kg.

12 noon: Beginning of diuresis control for 12 hours in a metabolic cage.

24h: Blood pressure, heart rate (30 min) recording, baroreflex sensitivity assessment, echocardiographic evaluation and left ventricular end-diastolic pressure measurement, and euthanasia to remove plasma for biochemical analysis and tissue for molecular biology analysis.

INFARCTION INDUCTION

The animals were weighed and anesthetized with a mixture of Ketamine (50 mg/Kg) and Xylazine (12 mg/Kg) intraperitoneally, placed in the supine position and intubated (gelco-14G). A small cut was made in the skin, and the pectoral muscles were pulled apart. The animal was submitted to artificial respiration (Intermed, Inter 3, São Paulo, SP) and a left thoracotomy was performed in the fourth intercostal space, with a retractor placed between the ribs for better visualization.

The pericardium was opened and the left atrium was moved away to visualize the anterior interventricular vein as a reference to the artery. The procedure for the ligation of the anterior interventricular artery consisted of the passage of a wire under the vein, reaching part of the

musculature, where the artery is located. This was ligated (6.0 mononylon thread) causing ischemia of the adjacent tissue.

After coronary ligation, the thoracotomy was closed (4.0 mononylon thread) and the pneumothorax was removed. The separated muscles were repositioned and the skin was sutured (4.0 mononylon thread). Soon after the animal's recovery and the onset of reflexes suppressed by the action of anesthetics, the animal was removed from artificial ventilation and breathed in a heated environment for recovery. It was administered intramuscularly with 30000 IU benzylpenicillin24**.** (Figure 1).

HEMODYNAMIC MEASUREMENTS

Blood pressure and heart rate measurements in rats 24 hours after surgical procedure induction (AMI);

The analysis of pressure signals was performed using a commercial program associated with the acquisition system. This program allowed the detection of maxima and minima of the pressure curve beat by beat, providing the systolic blood pressure (SBP) and diastolic blood pressure (DBP) values by the integral of the area under the curve in time. Heart rate (HR) was determined from the interval between two systolic peaks. The results were presented as mean values and standard deviations of the periods in which the data were analyzed for BP and HR. The data sheets obtained were analyzed in a commercial program for analysis (Excel 5.0), where the mean and standard deviation of BMP, SBP, DBP and HR were calculated for each animal. MAP variability was calculated using the mean of the standard deviations of each animal studied. The mean arterial pressure variability coefficient was obtained by means of the ratio of MAP variability to the mean arterial pressure value of each animal under study.[25th]

EVALUATION OF THE PRESSORECEPTOR REFLEX;

After MAP recording and the animals had remained at rest for 15 minutes, the sensitivity of the pressoreceptors was tested by infusion of phenylephrine and then sodium nitroprusside. Phenylephrine (Sigma Chemical Company, St. Louis, MO, USA), a potent α1 stimulator whose predominant action occurs in peripheral arterioles causing vasoconstriction, was injected in increasing doses into the femoral vein cannula. This drug was used, therefore, to cause an increase in blood pressure, an effect that causes subsequent reflex bradycardia, commanded by the pressoreceptors.

The opposite effect, i.e., a reduction in blood pressure with a tachycardia response, also commanded by the pressoreceptors, was caused by the injection of increasing doses of sodium nitroprusside (Sigma Chemical Company, St. Louis, MO, USA), a potent vasodilator of both

arterioles and veins, whose action occurs through the activation of guanylate cyclase and increased synthesis of 3', (minutes)- guanosine monophosphate (cyclic GMP) in the smooth muscle of vessels and other tissues.5'

To evaluate the sensitivity of the pressoreceptors, the maximum or minimum peak MAP was reduced from the MAP values of the control period. In the same way, the maximum variation of heart rate was reduced from the heart rate values of the control period, immediately before the infusion of the drugs, for later quantification of the responses. Baroreflex sensitivity was assessed by the index calculated by dividing the HR variation by the MAP variation.[25th]

STATISTICAL ANALYSIS

Data are expressed as mean ± standard error or mean ± standard deviation. Differences between the means of the multiple parameters were analyzed by the One-Way ANOVA method followed by the Newman-Keuls test. The statistical program used was GraphPrism 5.0. Values of $p < 0.05$ were considered statistically significant.

RESULTS

BIOCHEMICAL DATA

Table 1 below presents the biochemical data. As can be seen, there was a significant improvement in serum creatinine in AMI and AMI + C animals treated with 4F compared to animals with AMI and AMI+C not treated. Treatment with completely reversed this alteration.4F

Table 1 also shows an increase in serum levels of hepatic enzymes in animals infarcted with contrast, demonstrating hepatic dysfunction, with significant recovery of the alteration with the treatment of peptide 4F. Similarly, there was a significant increase in serum lactate in the AMI + C group. It is important to observe the levels of Triglycerides, Total Cholesterol, VLDL, LDL and HDL in animals with infarction. There is a significant increase in triglycerides, total cholesterol, VLDL and LDL, and a decrease in HDL in this model of contrast-enhanced AMI. Treatment with significantly reversed all changes, especially in the decrease in LDL and increase in HDL.4F4F

Table 1. Biochemical data in animals with 24-hour AMI or SHAM.

	SHAM	IAM	IAM+C	IAM+4F	IAM+C+4F
Creatinine (mg/dl)	0.27±0.02	0,57±0,04 [a,b,c]	0,65±0,04 [a,b,c]	0,38±0,02 [e]	0.39±0.02g
Urine volume (ml/12h)	0.02±0.002	0,006±0,001e,f	0,009±0,002f,k	0.02±0.005	0.03±0.004

Sodium excretion fractions (FENa %)	0.127±0.09	0.196±0.09	0.299±0.07	0.176±0.08	0.245±0.07
Potassium excretion fraction (FEK%)	14.9±1.88	19.6±8.44	27.0±7.96	22.2±8.07	22.8±8.35
Urinary osmolality (mOsm/kg)	860.1±84.6	1019.0±220.8	674.9±178.1	806.8±314.2	836.0±341.8
Lactate (mmol/l)	1.54±0.12	2,4±0,11 [j,k,l]	2,66±0,2 [g,h,l]	1.74±0.2	1.87±0.2
Triglicerídeos (Mg/dl)	39.8±8.5	114,1±14,6d,e,f	120,0±13,6d,e,l	43.9±11.6	62.58±13.6
Colesterol total (mg/dl)	95.7±7.5	145,0±10,5j,k,l	154,8±9,42g,h,i	93.8±11.4	100.9±14.0
VLDL (mg/dl)	7.1±0.8	23,7±0,95a,b,c	25,3±1,2a,b,c	8.62±2.1	11.1±2.8
LDL (mg/dl)	35.5±4.2	118.5±4.8a,b,c	120.9±5.4a,b,c	42.7±7.3j	62.2±5.2j
HDL (mg/dl)	44.0±4.3	57.5±10.7	42.3±9.9h,i	77.8±3.9g	72.6±4.1g

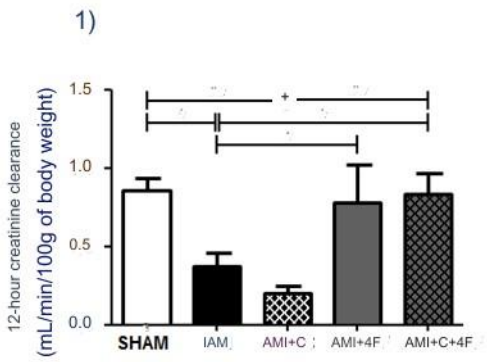
[a] $p < 0,0001$ vs. Sham; [b] $p < 0,0001$ vs. IAM+4F; [c] $p < 0,0001$ vs. IAM+C+4F; [d] $p < 0,001$ vs. Sham; [e] $p < 0,001$ vs. IAM+4F; [f] $p < 0,001$ vs. IAM+C+ 4F; [g] $p < 0,01$ vs. Sham; [h] $p < 0,01$ vs. IAM+4F; [i] $p < 0,01$ vs. IAM+C+4F; [j] $p < 0,05$ vs. Sham; [k] $p < 0,05$ vs. IAM+4F; [l] $p < 0,05$ vs. IAM+C+ 4F. Dados expressos em média ±EPM.

MEASUREMENTS OF RENAL FUNCTION THROUGH 12-HOUR CREATININE CLEARANCE;

As shown in Figure 1 and Table 2, we demonstrated that 24 hours after infarction induction, there is a significant reduction in 12-hour creatinine clearance in the 4F-treated groups.

Treatment with 4F restored renal function similar to the Sham group.

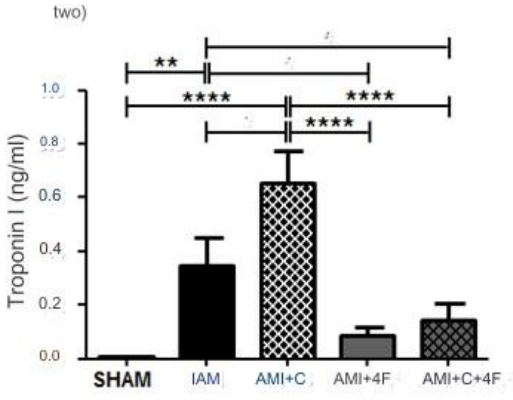
Figure 1. Creatinine clearance 12 hours after induction of AMI and AMI+C. *P≤0.05; **P≤0.01; P≤0.001; P≤0.0001(n=8 animals per group).



MEASUREMENTS OF CARDIAC FUNCTION AND THROUGH PLASMA TROPONIN I TESTING;

As can be seen in Figure 2A and Table 3, there is an increase in the ischemic area marker in the AMI and AMI+C groups. The AMI+4F and AMI+C+4F groups showed a significant reduction in troponin I values.

Figure 2. Plasma troponin I test 24 hours after MIC induction.

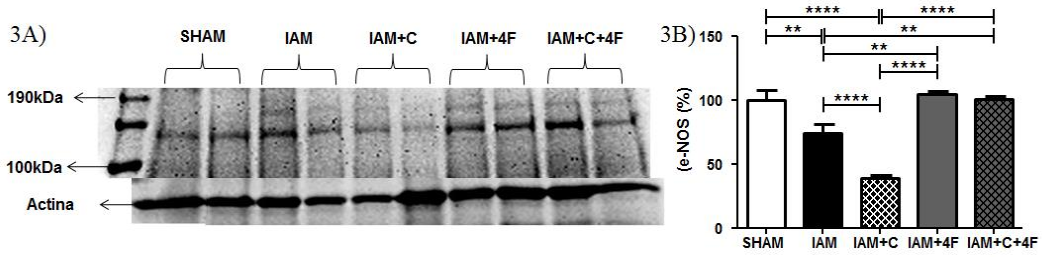


WESTERN BLOTTING STUDY FOR ANALYSIS OF PROTEIN EXPRESSION IN RENAL TISSUE

Study of eNOS expression;

As can be seen in (Figs 3A and 3B) and Table 4, there is a significant decrease in the expression of the eNOS protein. The expression of this protein in infarcted animals (AMI and AMI+C groups) is significantly lower. As in the SHAM group, treatment with the peptide restored eNOS expression in the AMI+4F and AMI+C+4F groups in renal tissue.

Figure 3A and 3B. Expression of eNOS protein in renal tissue. *P≤0.05; **P≤0.01; P≤0.001; P≤0.0001(n=8 animals per group).

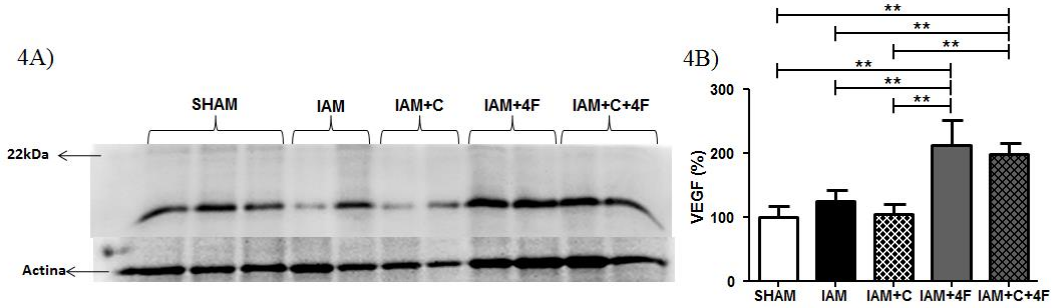


Study of VEGF expression;

As can be seen in (Figs 4A and 4B) and Table 5, there is a significant decrease in the expression of the VEGF protein. The expression of this protein in infarcted animals (AMI and AMI+C groups) was significantly lower, as well as in the SHAM group that did not receive ischemia

stimulation. Treatment with the peptide restored VEGF expression in the AMI+4F and AMI+C+4F groups in renal tissue.

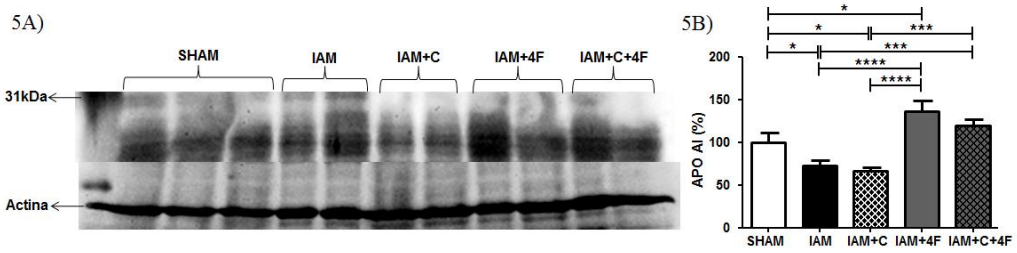
Figure 4A and 4B. Expression of VEGF protein in renal tissue. *P≤0.05; **P≤0.01; P≤0.001; P≤0.0001(n=8 animals per group).



Study of APO AI expression;

As can be seen in (Figs 5A and 5B) and Table 6, there is a significant decrease in the expression of the APO AI protein. The expression of this protein in infarcted animals (AMI and AMI+C groups) is significantly lower, as well as in the SHAM group that did not receive treatment. Treatment with the peptide restored the expression of APO AI in the AMI+4F and AMI+C+4F groups in renal tissue.

Figure 5A and 5B. Expression of APO AI protein in renal tissue. *P≤0.05; **P≤0.01; P≤0.001; P≤0.0001(n=8 animals per group).

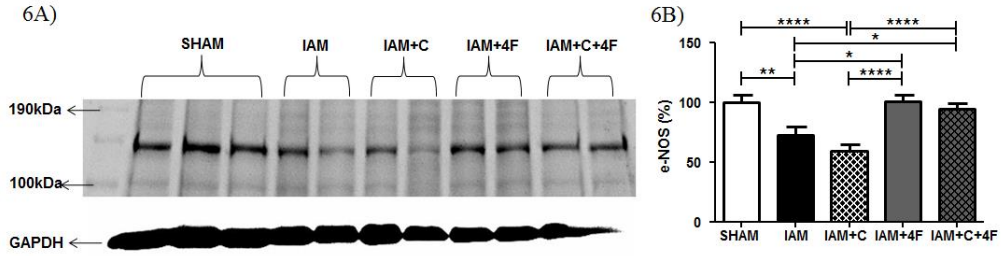


WESTERN BLOTTING STUDY FOR ANALYSIS OF PROTEIN EXPRESSION IN CARDIAC TISSUE;

Study of eNOS expression;

As can be seen in (Figs 6A and 6B) and Table 7, there is a significant decrease in the expression of the eNOS protein. The expression of this protein in infarcted animals (AMI and AMI+C groups) is significantly lower. As in the SHAM group, treatment with the peptide restored eNOS expression in the AMI+4F and AMI+C+4F groups in cardiac tissue.

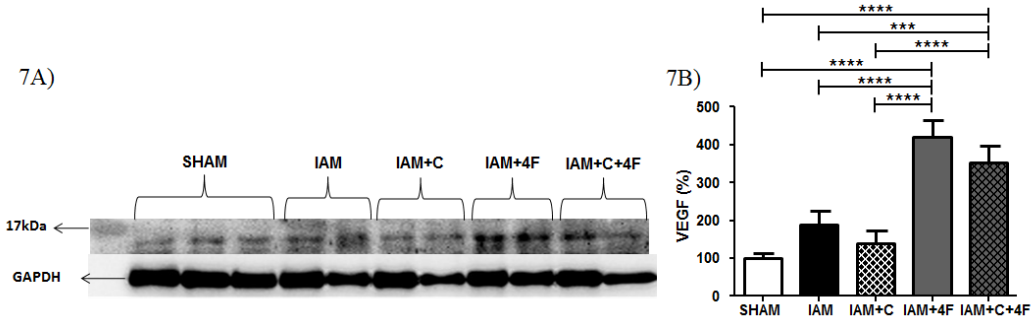
Figure 6A and 6B. Expression of eNOS protein in cardiac tissue. *P≤0.05; **P≤0.01; P≤0.001; P≤0.0001(n=8 animals per group).



Study of VEGF expression;

As can be seen in (Figs 7A and 7B) and Table 8, there is a significant decrease in the expression of the VEGF protein. The expression of this protein in infarcted animals (AMI and AMI+C groups) was significantly lower, as well as in the SHAM group that did not receive ischemia stimulation. Treatment with the peptide restored VEGF expression in the AMI+4F and AMI+C+4F groups in cardiac tissue.

Figure 7A and 7B. Expression of the VEGF protein in cardiac tissue. *P≤0.05; **P≤0.01; P≤0.001; P≤0.0001(n=8 animals per group).

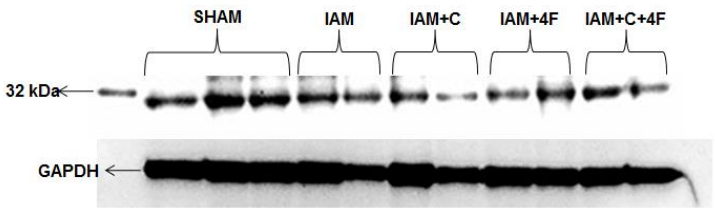


Study of APO AI expression;

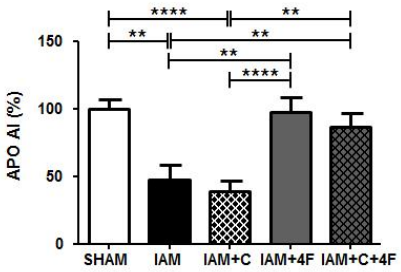
As can be seen in (Figs 8A and 8B) and Table 9, there is a significant decrease in the expression of the APO AI protein. The expression of this protein in infarcted animals (AMI and AMI+C groups) is significantly lower, as well as in the SHAM group that did not receive treatment. Treatment with the peptide restored APO AI expression in the AMI+4F and AMI+C+4F groups in cardiac tissue.

Figure 8A and 8B. Expression of APO AI protein in cardiac tissue. *P≤0.05; **P≤0.01; P≤0.001; P≤0.0001(n=8 animals per group).

8A)



8B)



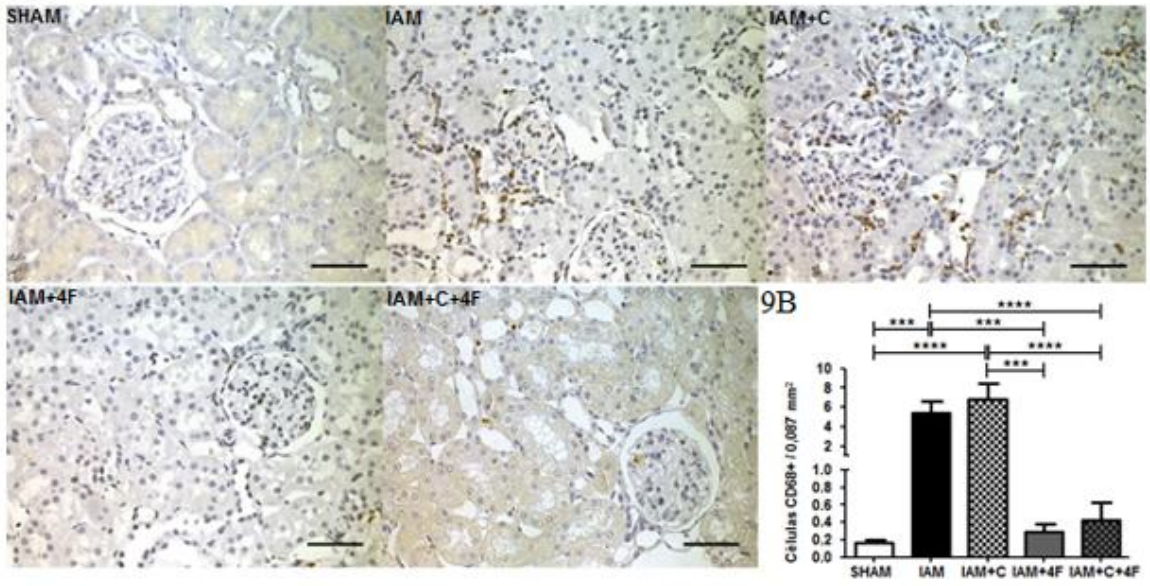
IMMUNOHISTOCHEMISTRY STUDY FOR THE DETERMINATION OF PROTEIN EXPRESSION OF CELLS AND MITOCHONDRIAL MORPHOLOGY IN MYOCARDIAL INFARCTION IN RENAL TISSUE;

Study of the protein expression of CD68-positive cells in renal tissue;

We can observe an increase in macrophages in the AMI and AMI+C groups in the (Fig. 9A, 9B and Table 10) showed a more expressive positive staining score and there was a significant improvement in the AMI+4F and AMI+C+4F groups represented in (Figs 9A, 9B and Table 10) *compared to the SHAM group.*

Figure 9A and 9B. Immunohistochemistry of CD68 expression in renal tissue. *P≤0.05; **P≤0.01; P≤0.001; P≤0.0001(n=6 animals per group).

9A

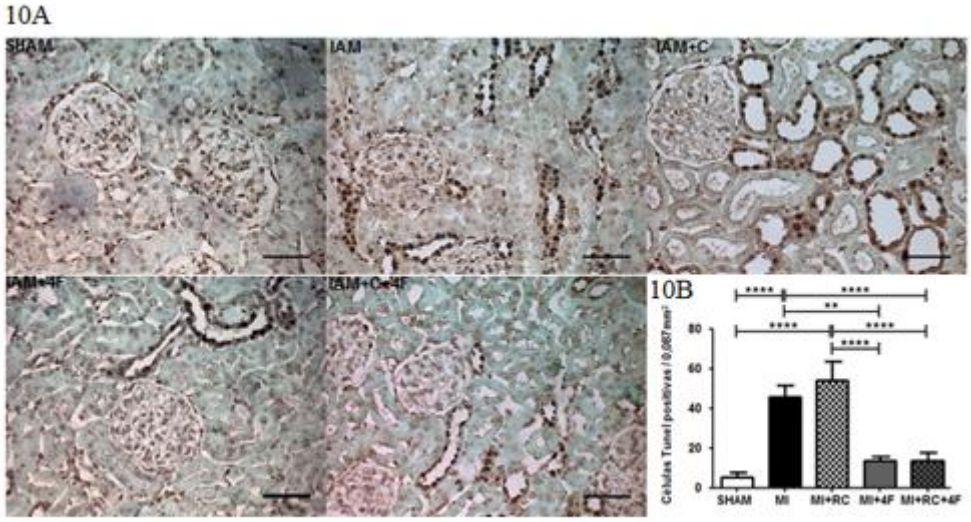


Study of the protein expression of positive Tunel cells in renal tissue;

We can observe an increase in the number of apoptotic cells in the AMI and AMI+C groups in the (Fig. 10A, 10B and Table 11) showed a more expressive positive staining score and there was a

significant improvement in the AMI+4F and AMI+C+4F groups represented in (Figs 10A, 10B and Table 11) *compared to the SHAM group.*

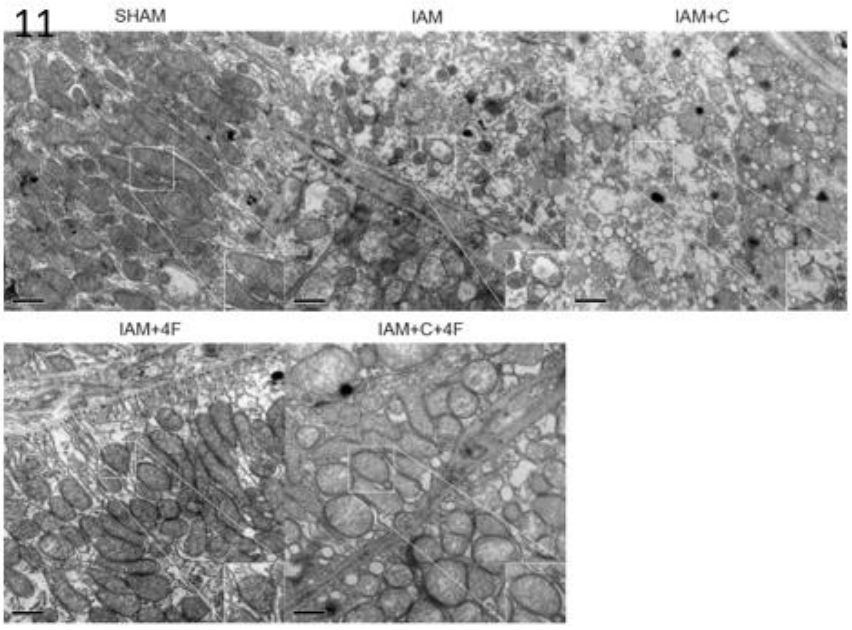
Figure 10A and 10B. Immunohistochemistry of the expression of positive tunnel cells in renal tissue. *P≤0.05; **P≤0.01; P≤0.001; P≤0.0001(n=6 animals per group).



Study of mitochondrial morphology in myocardial infarction in renal tissue;

We can observe morphological changes in the crests of the mitochondria with balloon formations within their structure in the AMI and AMI+C groups in the (Fig. 11) showed a visible alteration in the image. We can associate the same alterations found in the mitochondria of the heart and with the treatment there was an improvement in the alterations in the AMI+4F and AMI+C+4F groups, which had morphological aspects more similar to the SHAM group represented in (Fig. 11).

Figure 11. Mitochondrial morphology in myocardial infarction in renal tissue.

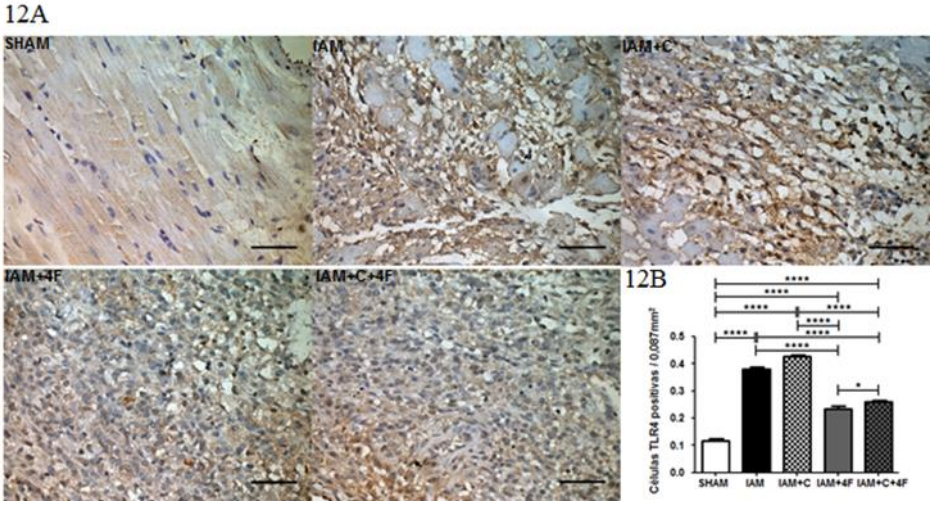


IMMUNOHISTOCHEMISTRY STUDY FOR THE DETERMINATION OF PROTEIN EXPRESSION OF CELLS AND MITOCHONDRIAL MORPHOLOGY IN MYOCARDIAL INFARCTION IN CARDIAC TISSUE;

Study of the protein expression of TLR4 positive cells in cardiac tissue;

We can observe an increase in the number of positive TLR4 cells in the AMI and AMI+C groups in (Figs 12A, 12B and Table 12) showed a more expressive positive staining score and there was a significant improvement in the AMI+4F and AMI+C+4F groups represented in (Figs 12A, 12B and Table 12) *compared to the SHAM group.*

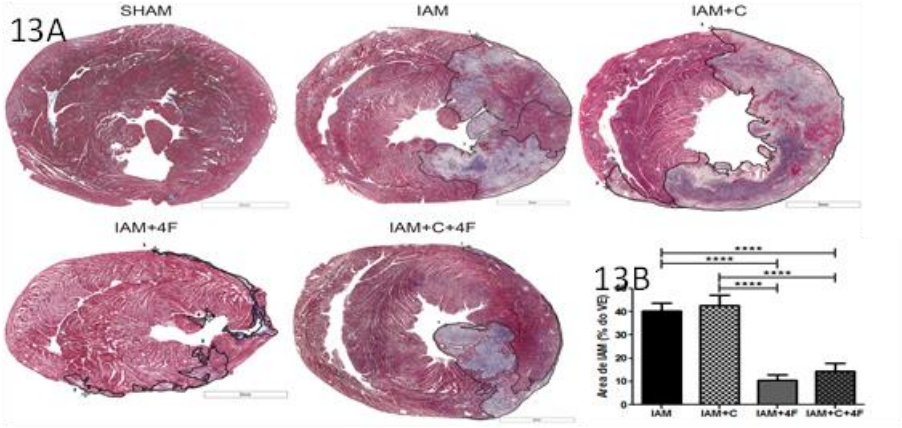
Figure 12A and 12B. Immunohistochemistry of TLR4-positive cell expression in cardiac tissue. *P≤0.05; **P≤0.01; P≤0.001; P≤0.0001(n=6 animals per group).



Study of infarct area expression in cardiac tissue;

Figs 13A, 13B and Table 13 show images of cross-sectional sections of the hearts of the groups studied, at the level of the papillary muscles, as measured by the ratio between the volume of the area marked with Masson's dye and the total volume of the heart. The percentage of infarct area is increased in the AMI and AMI+C groups, and the animals treated with 4F (AMI+4F and AMI+C+4F) showed a significant improvement in the infarct area.

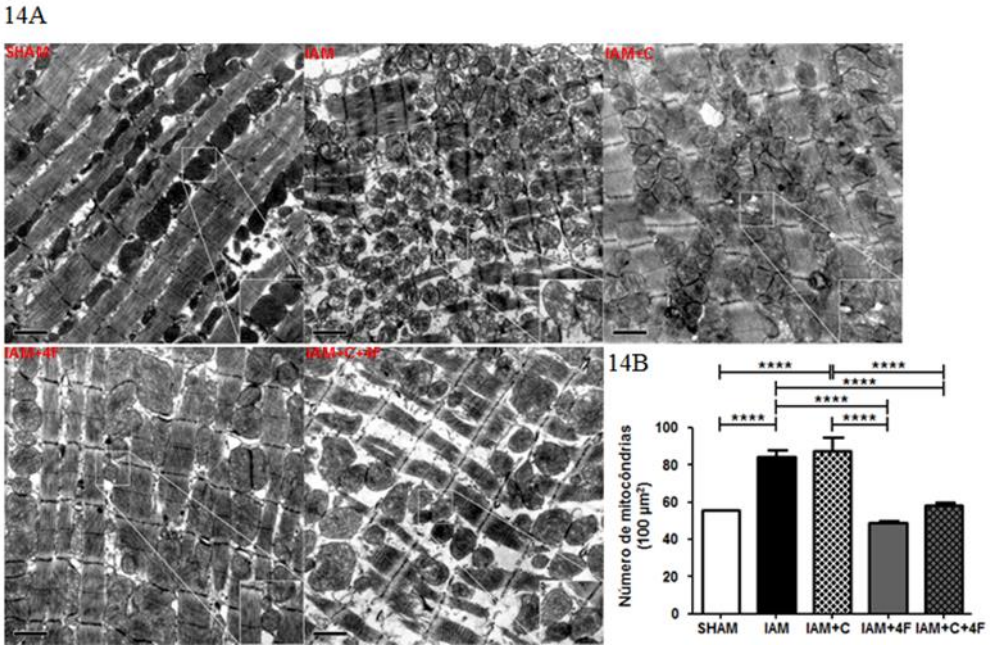
Figure 13A and 13B. Immunohistochemistry of AMI area expression in cardiac tissue. *P≤0.05; **P≤0.01; P≤0.001; P≤0.0001(n=6 animals per group).



Study of mitochondrial morphology and quantification in myocardial infarction in the left ventricular region in rat hearts;

As can be seen in (Fig. 14A, 14B and Table 14), there was an increase in the number of mitochondria identified by the electron microscopy technique in infarcted animals in the AMI and AMI+C groups. There was a significant improvement in the AMI+4F and AMI+C+4F groups represented in (Figs 14A, 14B and Table 14) *compared to the SHAM group.* The result found in our model of cardiac dysfunction corroborates the picture presented so far and reinforces the premise that modifications in mitochondrial structure are associated with an increase in mitochondria in the infarcted area and compromising the proper functioning of the heart pump.

Figure 14A and 14B. Number of mitochondria (100 µm2 mitochondrial area). *P≤0.05; **P≤0.01; P≤0.001; P≤0.0001 (n=6 animals per group).

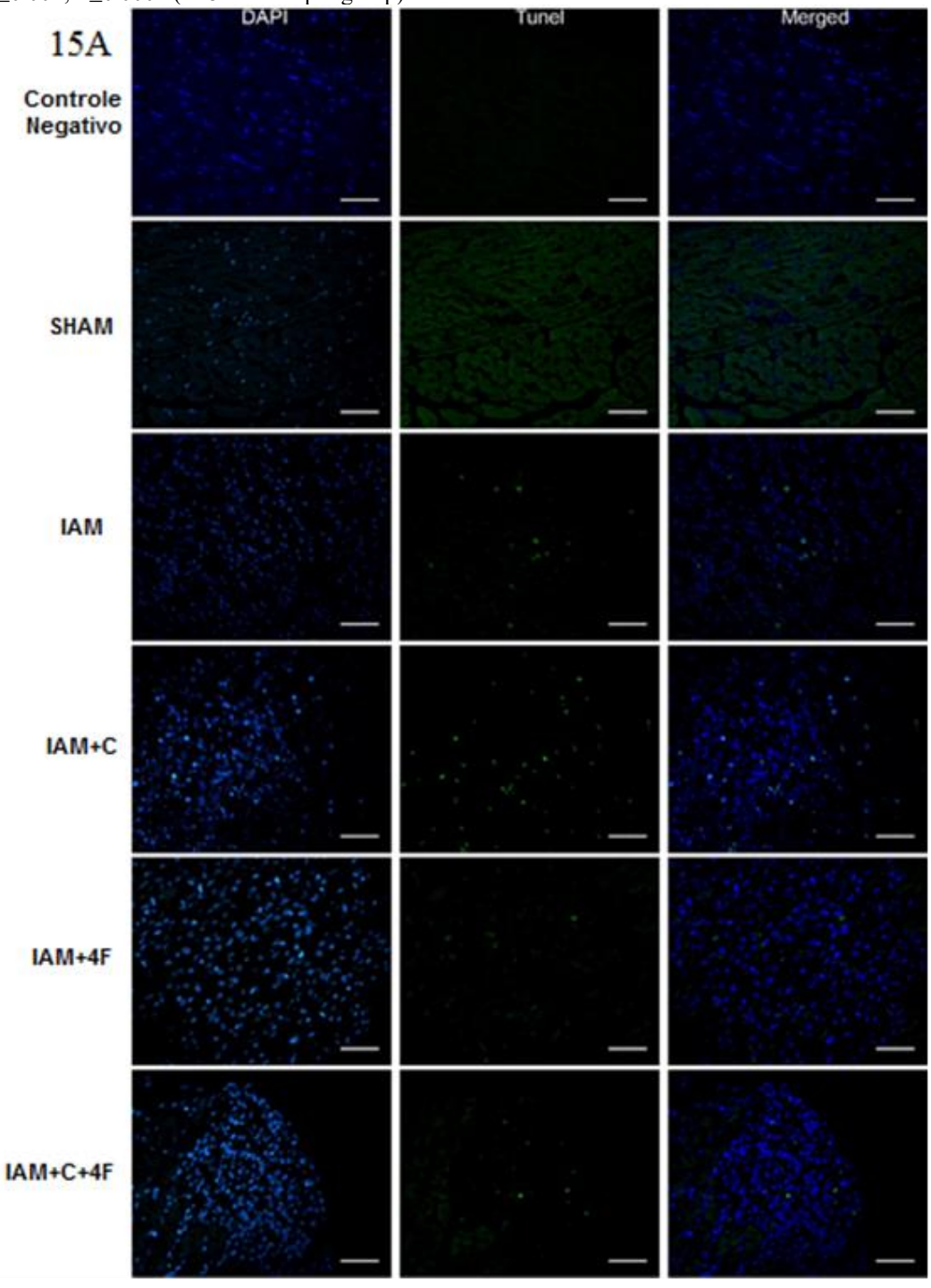


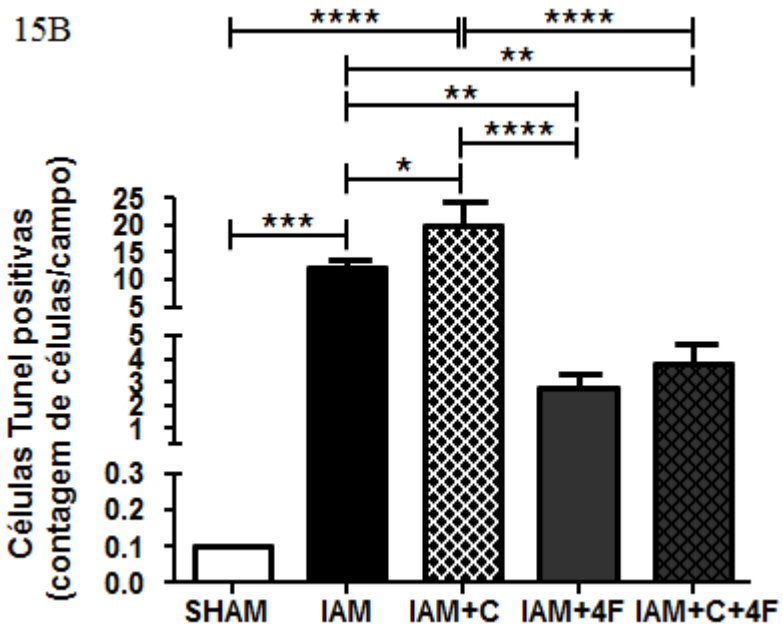
IMMUNOFLUORESCENCE STUDY FOR THE DETERMINATION OF PROTEIN EXPRESSION OF CELLS IN CARDIAC TISSUE

Study of the protein expression of positive Tunnel cells in cardiac tissue;

We can observe an increase in the number of apoptotic cells in the AMI and AMI+C groups in the (Fig. 15A, 15B and Table 15) showed a more expressive positive staining score and there was a significant improvement in the AMI+4F and AMI+C+4F groups represented in (Figs 15A, 15B and Table 15) *compared to the SHAM group.*

Figure 15A and 15B. Immunohistochemistry of the expression of positive tunnel cells in cardiac tissue. *P≤0.05; **P≤0.01; P≤0.001; P≤0.0001 (n=8 animals per group).

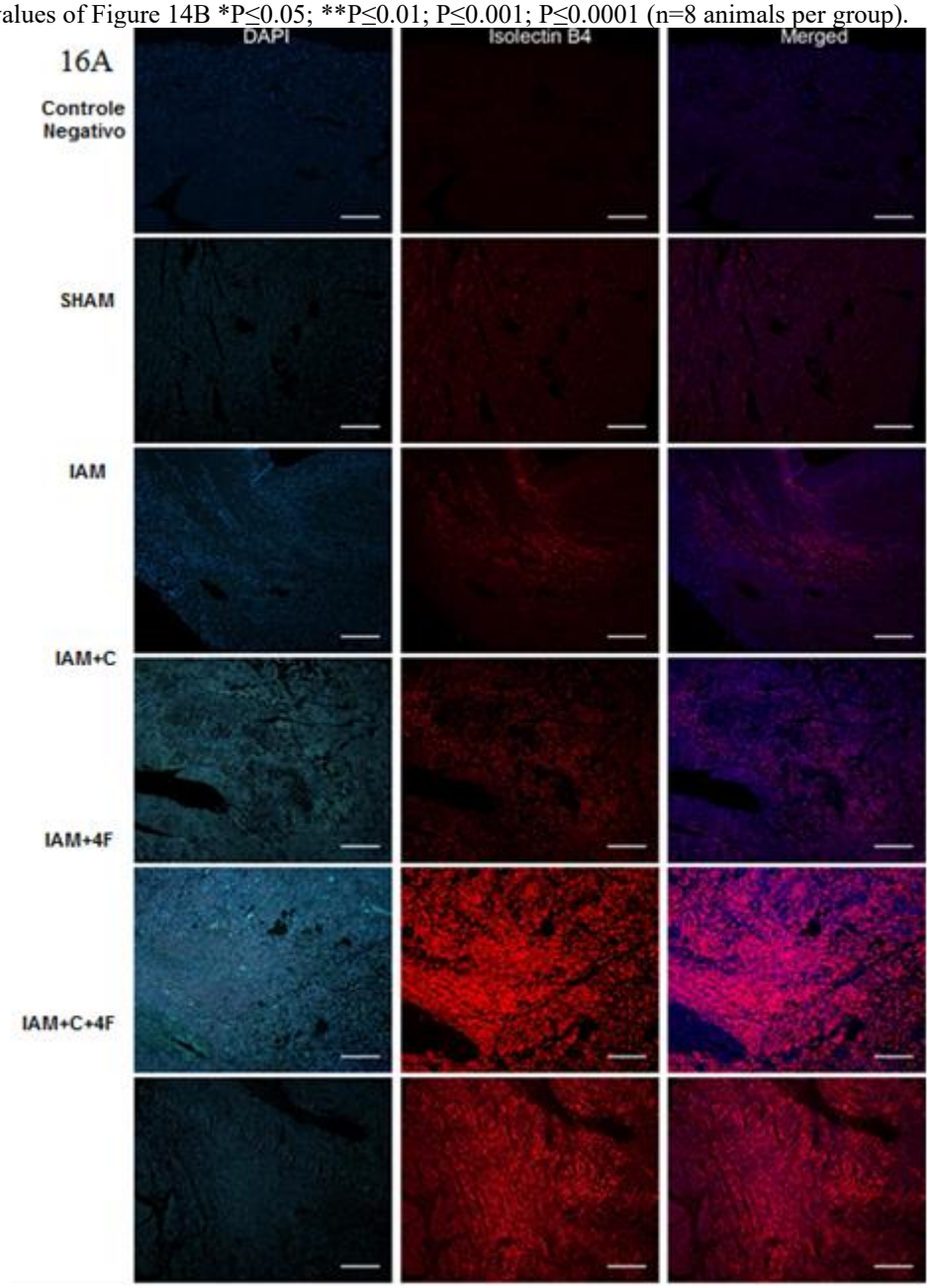


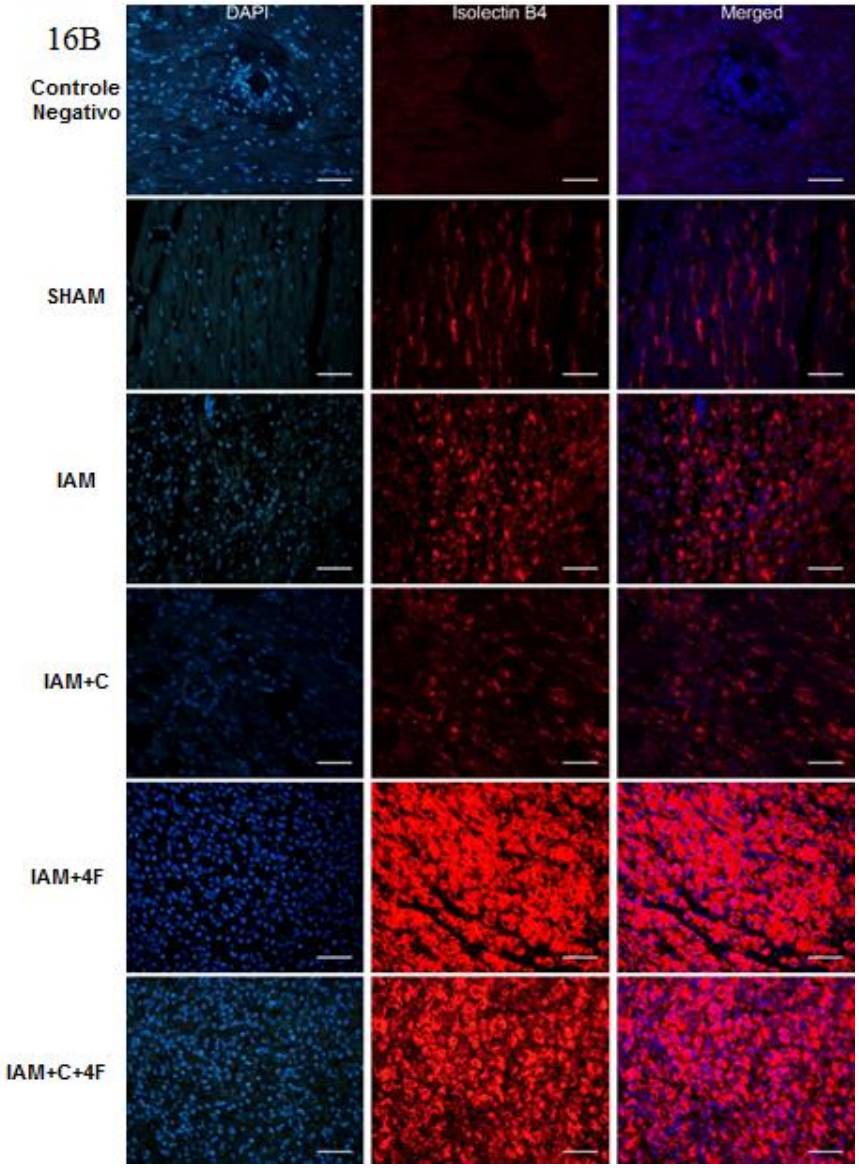


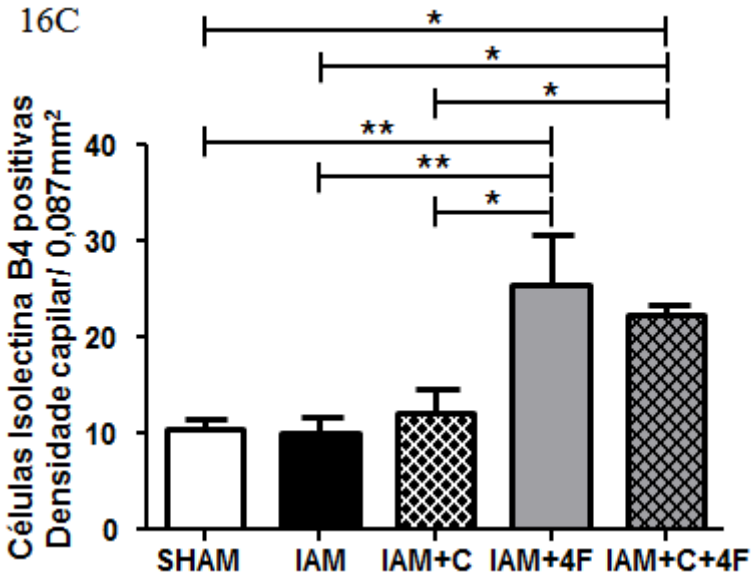
Study of the protein expression of B4-positive isolectin cells in cardiac tissue;

We can observe a decrease in angiogenesis in the AMI and AMI+C groups in the (Fig. 16A, 16B, 16C and Table 16) showed a decrease in the expression of capillary microvascularization, representative through the score with decreased coloration, and there was a significant improvement in the AMI+4F and AMI+C+4F groups represented in (Figs 16A, 16B, 16C and Table 16) *compared to the SHAM group.*

Figure 16A, 16B, and 16C. Immunohistochemistry of the expression of Isolectin B4 positive cells in cardiac tissue. Representative values of Figure 14B *P≤0.05; **P≤0.01; P≤0.001; P≤0.0001 (n=8 animals per group).




16B
DAPI
Isolectin B4
Merged
Controle Negativo
SHAM
IAM
IAM+C
IAM+4F
IAM+C+4F


16C
Células Isolectina B4 positivas
Densidade capilar/ 0,087mm²
0
10
20
30
40
*
*
*
**
**
*
SHAM
IAM
IAM+C
IAM+4F
IAM+C+4F

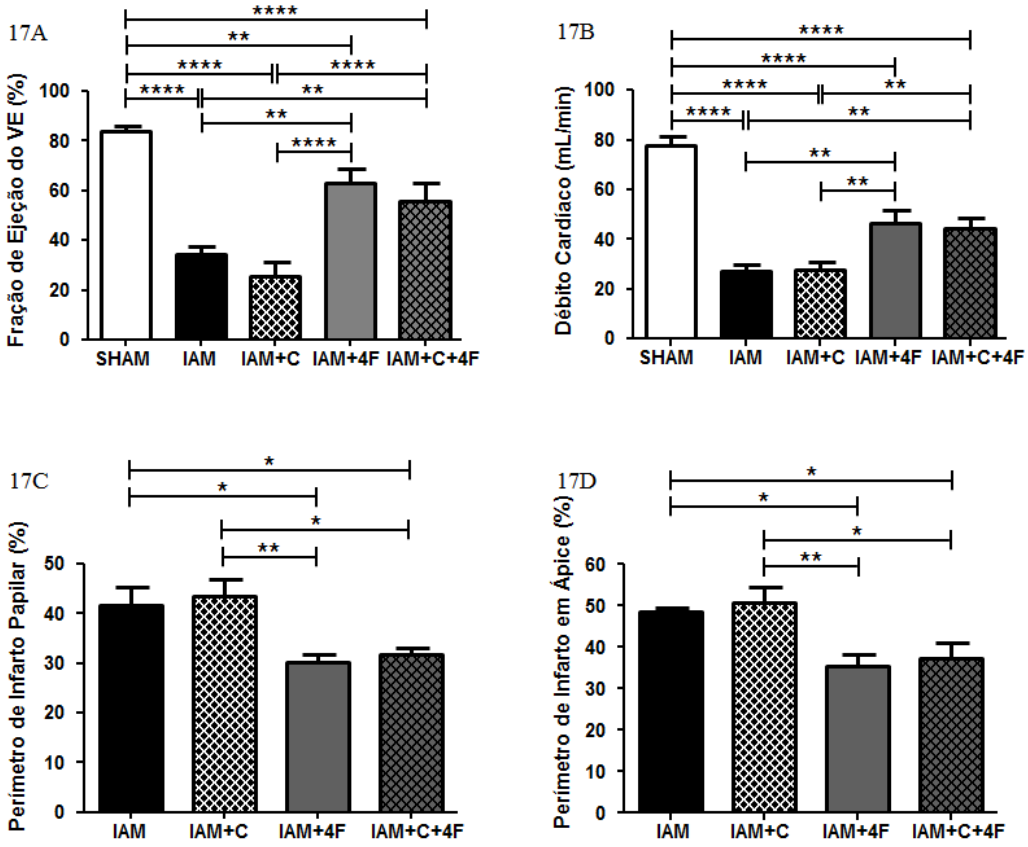
CARDIAC FUNCTION AND HEMODYNAMIC MEASUREMENTS

Measurements of cardiac function and echocardiogram at 24 h after AMI induction;

As can be seen in Figures 17A and 17B, Table 17, there was a significant reduction in ejection fraction and cardiac output in the AMI and AMI+C groups.

As observed in the AMI+4F and AMI+C+4F groups in Figures 17A, 17B and Table 17, the treatment significantly improved ventricular function. The infarct area was measured in the apex and papillary region of the myocardium by echocardiogram in figures 17C, 17D and table 17, showing an improvement in the vascularization of the ischemic area in the AMI+4F and AMI+C+ groups. 4F

Figure 17A, 17B, 17C, and 17D. Echocardiogram 24 hours after AMI induction. *P≤0.05; **P≤0.01; P≤0.001; P≤0.0001(n=8 animals per group).

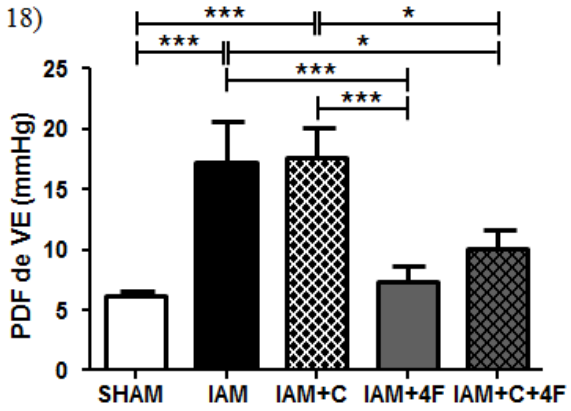


[a] p < 0,0001 vs. Sham; [b] p < 0,0001 vs. IAM+4F; [c] p < 0,0001 vs. IAM+C+4F; [g] p < 0,01 vs. Sham; [h] p < 0,01 vs. IAM+4F; [i] p < 0,01 vs. IAM+C+4F; [k] p < 0,05 vs. IAM+4F; [l] p < 0,05 vs. IAM+C+ 4F. Dados expressos em média ±EPM.

Cardiac function measurements using direct LVPDF measurement at 24 h after AMI induction;

LV DIS was increased in the AMI and AMI+C groups compared to the Sham group. This pressure normalized with treatment in the AMI+4F and AMI+C+4F groups, shown in Figure 18 and Table 18.

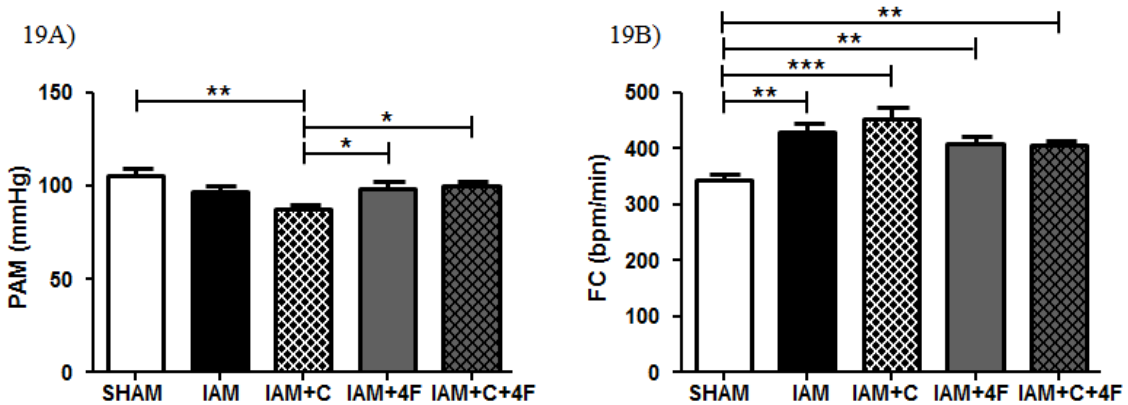
Figure 18. Measurement of LVPD 24 hours after MAI induction. *P≤0.05; **P≤0.01; ***P≤0.001; ****P≤0.0001(n=8 animals per group).



MAP and HR measurements 24 h after AMI induction;

When we compare MAP 24 h after AMI induction in Figure 19A and Table 19, we observed a statistical difference in the AMI+C group, showing a significant decrease in MAP that is already expected in this infarction model, and the AMI group did not show any difference. However, it was recovered in the AMI and AMI+C+4F groups together with the Sham group. However, the AMI, AMI+C groups did not show any difference in comparison with the AMI+4F and AMI+C+4F groups, but there was a statistical difference between the groups without and with treatment compared to the Sham group, showing an increase in HR after AMI induction in Figure 19B and Table 19. Therefore, we found that the AMI+C group showed a decrease in MAP associated with an increase in HR.

Figure 19A and 19B. Hemodynamic evaluation of MAP and HR 24 hours after MAI induction. *P≤0.05; **P≤0.01; ***P≤0.001; ****P≤0.0001(n=8 animals per group).



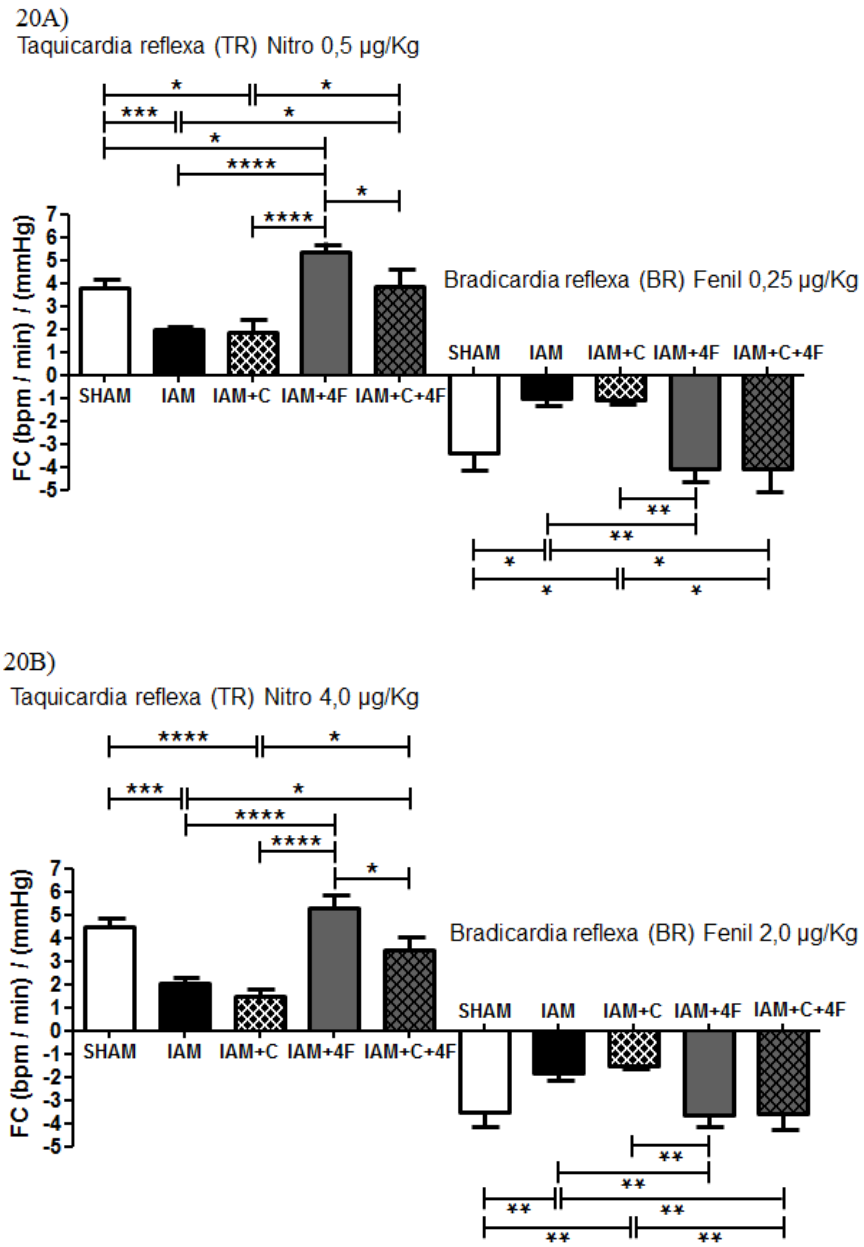
Measures of baroreceptor response 24 h after AMI induction;

In the study of the response of the baroreceptors, we obtained extremely interesting data. Reflex tachycardia induced by nitroprusside, both at low dose 0.5 µg/kg (Fig. 20A) and at high dose

4.0 µg/kg (Fig. 20B), is altered in the AMI and AMI+C groups. Treatment with 4F re-established this baroreceptor response (Fig. 20A, 20B and Table 20).

The same is explained for the study of the response of baroreceptors to a bradycardizing stimulus. Phenylephrine-induced reflex bradycardia at both low 0.25 µg/kg (Fig. 20A) and high dose 2.0 µg/kg (Fig. 20B) is altered in animals with AMI and AMI+C. In animals treated with 4F, this response is recovered (Fig. 20A, 20B and Table 20).

Figures 20A and 20B. Study of the response of baroreceptors 24 hours after MAI induction. *P≤0.05; **P≤0.01; ***P≤0.001; ****P≤0.0001(n=8 animals per group).



DISCUSSION

This is the first study to characterize the effects of apolipoprotein AI on renal and cardiac dysfunction after myocardial infarction in hypercholesterolemic rats receiving contrast.

MODEL OF CARDIAC AND RENAL DYSFUNCTION ASSOCIATED WITH MYOCARDIAL INFARCTION

Knowing that cardiac surgery to induce myocardial infarction in animals is used to mimic the ventricular dysfunction observed in humans, and in order to confirm cardiac dysfunction in the proposed animal model, an initial characterization of the phenotype of animals 24 hours after anterior descending coronary artery ligation surgery was part of this study. To this end, we evaluated cardiac and physiological morphofunctional parameters in the groups studied.[17, 18, 81]

CARDIAC MORPHOFUNCTIONAL CHANGES AND PHYSIOLOGICAL PARAMETERS IN AN ANIMAL MODEL OF CARDIAC DYSFUNCTION ASSOCIATED WITH MYOCARDIAL INFARCTION

Initially, cardiac function and structure were evaluated by means of echocardiographic examination 24 hours after myocardial infarction induction. As observed in the results, 24 hours after coronary artery ligation surgery, the AMI and AMI+C groups showed a reduction ejection fraction, cardiac output, and increased infarct area contributing to ventricular dysfunction. However, there was a significant improvement in the animals treated with 4F, the use of echocardiography in the cardiac morphofunctional evaluation is highly recommended for prognostic purposes and as indicators of ventricular dysfunction progression and its variables are used for the identification of the syndrome in both humans and rodents. [17, 83]

We believe that the left ventricular dysfunction observed in these animals was a consequence of the exacerbated loss of cardiac contractile tissue due to the ischemic process, since after surgery the animals presented an infarcted area of around 40% of the total cardiac area.[18, 84]

In addition to the echocardiographic alterations, the AMI and AMI+C groups showed an increase in the left ventricular (LV) end-diastolic pressure (PDF), which has a significant hemodynamic importance because it is measured within the left ventricular cavity of infarcted animals and records the ventricular filling pressures that determines hemodynamic changes and was reversed with the treatment of 4F in the AMI+4F and AMI+C+4F groups.[85]

We also evaluated troponin I in plasma, which is a gold standard marker of ischemic myocardial injury. The results showed a significant change in the animals infarcted without treatment (AMI and AMI+C) compared to the groups that received the treatment (AMI+4F and AMI+C+4F).

The efficacy of the method was confirmed when compared with the sham group, which did not show changes in plasma levels.[19]

The results suggest that decreased cardiac output is associated with reduced effective arterial volume of organs. Failure of cardiac performance, as seen in animals with heart failure, contributes to worsening survival in infarcted patients.[31] Apolipoprotein AI restored cardiac performance (improving cardiac output, ejection fraction, infarction area, LV PDF, and troponin I).

It is important to report that, associated with these cardiac alterations, we found an increase in the expression of TLR4-positive cells in the heart, as seen by immunohistochemistry, and associated with a decrease in the expression of endothelial synthesa nitric oxide (eNOS) proteins and an increase in lactate in the animals of the AMI and AMI+C groups, signaling an increase in inflammation in the cardiac tissue. The same is observed in other studies with worsening of cardiac function, but the animals treated with AMI+4F and AMI+C+4F showed a significant improvement in this marker.[75, 86-89]

The alterations studied in the heart constitute the basis for a better understanding of the effects resulting from myocardial infarction, since the mitochondria are a fundamental part in the maintenance of the viability and functionality of the cardiomyocyte.

As described in the results, we found changes in the morphology of the organelle, represented by the greater number of mitochondria with decreased muscle fibers in the hearts of AMI and AMI+C animals. There are studies correlating a reduction in the expression of the Parkin protein with mitochondrial autophagy (increase in the amount of mitochondria with reduced area) observed by the electron microscopy technique in infarcted animals.[90, 91]

The results corroborate and indicate that impairments in mitochondrial function cannot be compensated by the increase in the number of mitochondria, and that an appropriate balance in the structure of the organelle is necessary for the maintenance of cardiac homeostasis in infarcted rats.

Energy production in heart tissue is not only related to mitochondrial morphology, but is also involved in the structural organization of organelles, represented by density and location. Due to the continuous energy demand generated by ATP, adult cardiomyocytes have extremely dense mitochondria when compared to other tissues, since in addition to effectively participating in the synthesis of ATP via oxidative phosphorylation, the organelle also participates in Ca2+ signaling, cell proliferation and apoptosis.[92-94]

When we investigated the apoptosis pathway through the expression of positive tunnel by immunofluorescence in the cardiac tissue, we observed an increase in the AMI and AMI+C groups, which is associated with the increase of VEGF protein by western blot and decrease in angiogenesis represented by the expression of B4 isalectin in cardiac tissue in immunofluorescence. However, the

groups that received 4F treatment (AMI+4F and AMI+C+4F) showed significant improvement in all these markers of apoptosis in the cardiac tissue.[95-98]

Taken together, our data demonstrate an important contribution of cardiac function attributed to the treatment of 4F that was confirmed by the significant increase in the expression of apolipoprotein AI in the cardiac tissue of the AMI+4F and AMI+C+4F groups by western blot. Our lipid profile data report a significant difference in the increase in HDL, decrease in LDL, decrease in VLDL and plasma triglycerides of the treated animals when compared with the AMI, AMI+C groups.[46, 66, 74, 99]

RENAL ALTERATIONS AND PHYSIOLOGICAL PARAMETERS IN AN ANIMAL MODEL OF CARDIAC DYSFUNCTION ASSOCIATED WITH MYOCARDIAL INFARCTION

In this study, a significant reduction in glomerular filtration measured by creatinine clearance associated with reduced urine flow was observed in the AMI and AMI+C groups, which was completely reversed in the AMI+4F and AMI+C+4F groups that received the treatment. However, the groups treated even with a significant increase in urinary flow did not show significant differences in sodium excretion fractions, potassium excretion fractions and urinary osmolality 24 hours after infarction induction.[8]

It is important to emphasize that associated with the decrease in glomerular filtration, there were some renal structural alterations, such as: increase in CD68 positive cells by immunohistochemistry associated with decreased expression of eNOS protein by western blot and increase in plasma lactate as was reported in cardiac alterations. We can affirm that there was a cross-tok between the heart and the renal system through an inflammatory interaction that was demonstrated by significant alterations in the AMI and AMI+C groups when compared to the SHAM, AMI+4F and AMI+C+4F groups.[72, 100]

In addition, there is also an important protein impairment related to the vascular integrity of the endothelium, which could explain the endothelial dysfunction occurring after ischemia and being considered as a consequence of impaired NO release (which is also observed in infarction patients). Our hypothesis is based on the alteration of the protein responsible for endothelial integrity (e-NOS). All of these events would be associated with a decrease in apo AI in renal tissue.[101]

We believe that the findings of renal alterations are also related to inflammation, oxidative stress, cell proliferation and apoptosis similar to cardiac abnormalities. Our results demonstrate a significant increase in the expression of positive tunnel cells by immunohistochemistry, increased expression of VEGF protein by western blot and associated with morphological changes of mitochondria that are related to cell death by apoptosis in the AMI and AMI+C groups. These results may contribute to a better understanding of acute kidney injury in cardiac events.[102-106]

The results of the present study show that the treatment with 4F was positive in the renal tissue, showing a significant increase in the expression of apolipoprotein AI in the AMI+4F and AMI+C+4F groups compared to the animals in the SHAM, AMI and AMI+C groups. Improving or Cardiac performance and renal function in contrast-infarcted rats. [46, 66, 74, 99]

HEMODYNAMIC CHANGES IN THE ANIMAL MODEL OF CARDIAC DYSFUNCTION ASSOCIATED WITH MYOCARDIAL INFARCTION

The response of the baroreceptors is also altered. There was an alteration in MAP in animals with AMI+C associated with an increase in HR in relation to sham and treated animals. However, the AMI group did not show significant changes. Studies have shown that these changes in baroreceptor response are associated with increased morbidity.[107]

Reduced HDL is correlated with impaired cardiac function in patients with systemic inflammatory response syndrome.[74, 108]. In our study, animals with AMI and AMI+C, the LDL was significantly increased. Nature pro-inflammatory LDL has been attributed to the cytotoxic release and lipid peroxidation.[46] Oxidized LDL alters the endothelium and leads to its dysfunction. Recently, they have shown that peptide 4F prevents lipid peroxidation.[46] Data suggest that it induces the formation of new HDL particles that are enriched in paraoxonase, an enzyme that degrades lipid peroxides.4F[69]

Administration of peptide 4F increases paraoxonase activity, decreases cytokine synthesis and release, and decreases oxidative stress. [69, 109]

In a recent study, it was shown that the HDL in the normal range appears to be completely protective against klotho protein dysfunction.[110] With aging, plasma HDL concentrations decrease.[110] Decreased serum HDL concentration and function may occur secondary to hormonal changes, inflammatory processes, and diabetes mellitus.[110, 111] HDL deficiency is extremely rare among centenarians. HDL can modulate the aging process, not only because of its well-known activity antiatherogenic, but possibly also by directly interfering with aging by signaling the klotho protein. Most of the current results, however, are based on cell culture. There is no confirmation yet *in vivo*.[58]

In an extremely aggressive disease such as infarction, treatment with 4F peptide can be a great therapeutic option.[76]

The baroreflex system is responsible for maintaining cardiovascular homeostasis and preserving blood flow to vital organs.[112] It is known that in infarction, there is a direct relationship between baroreceptor sensitivity and survival, which is diminished when there is dysfunction of the baroreflex response.[107]. It has been described that eNOS activity is decreased in endothelial cells when exposed to LDL.[113] It is also described that oxidized LDL can alter the expression of

eNOS.[114] Alterations in HR and MAP variability, determined in part by nitric oxide-dependent endothelial dysfunction, are related to an adverse prognosis in cardiovascular diseases. Pelet et al. demonstrated that rosuvastatin decreases the expression of caveolin-1 (an eNOS inhibitor) and promotes improved eNOS function in dyslipidemic mice *knockouts* for apo E, with improvement in HR and MAP variability in these animals.[115] We demonstrated a dysfunction of the baroreceptor system in animals with AMI and AMI+C. Further studies will need to be done to identify whether the improvement in baroreflex sensitivity in animals treated with apolipoprotein AI is due to an increase in eNOS expression. In addition, this improvement may also be due to increased serum HDL levels and decreased serum LDL levels.

The mortality of patients with cardiovascular disease associated with acute kidney injury affects a large part of the population.[8]

In our study, there was normalization of renal function (measured by creatinine clearance) with treatment with apolipoprotein AI.

Therefore, treatment with Apo AI 4F, leads to anti-inflammatory effects, improves heart and kidney function. We also demonstrated that, in this infarction model, there is a dysfunction of the baroreflex response. The treatment, associated with an increase in HDL levels, probably led to a protection of the endothelium. This endothelial protection can be interpreted by the best expression of eNOS.

CONCLUSION

Our results demonstrate that the use of contrast in hypercholesterolemic animals with AMI greatly contributes to the worsening of cardiac and renal dysfunction.

And based on the results, we can affirm that 4F, through an HDL-dependent pathway, currently used as a therapeutic strategy in the treatment of cardiovascular diseases, is capable of reversing the changes in the cardiac and renal function of infarcted animals. Thus, we conclude that 4F plays an important role in protection, with beneficial effects on the maintenance of endothelial integrity, improvement of myocardial contractile function and renal system. Therefore, finding the most effective way to treat 4F in cardiovascular and renal diseases represents an important step for the future of research in the treatment of cardiorenal syndrome.

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