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Angiotensin- (1-7) actions on the proliferation of cardiomyocytes and Cardiac regeneration

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Received: / Accepted: / Published:

Abstract: Cardiovascular diseases represent a major public health problem and are the leading causes of death worldwide, with ischemic heart disease leading the causes of death worldwide. In general, the heart will suffer an injury, a heart attack, which will heal through a fibrous tissue that will serve as a mechanical support to prevent rupture of the cardiac wall, causing changes in the architecture and ventricular geometry, which may lead to insufficiency Cardiac. The major problem in this regard is that adult cardiomyocytes do not proliferate spontaneously, however, studies have demonstrated that cardiomyocyte proliferation and cardiac regeneration are possible. Data obtained by our research group indicate that angiotensin- (1-7), an endogenous peptide, has the potential to induce cardiomyocyte proliferation; The central objective is to investigate whether Ang- (1-7) promotes cardiomyocyte proliferation and cardiac regeneration. For this, Wistar rats 250-300g and Approved by the Committee on Ethics in the Use of Animals of the Center of Biotechnology of the Federal University of Paraíba, under the protocol CEUA nº 0204/13. Two measures of cardiac hypertrophy were used. The first is the ratio of the weight of the heart to the length of the tibia, and the second is the ratio of the weight of the heart to the body weight. As a result, the first reason did not demonstrate any anti-hypertrophic response. In the second reason, the result was satisfactory. The method of enzymatic dissociation of cardiac tissue was used. The method is based on retrograde perfusion through the aortic artery using a collagenase-containing digestion solution. Thus, we conclude that the results indicate an antihypertrophic effect of Ang- (1-7) and that RNA extractions were satisfactory in order to obtain materials with good quantity and quality.

Keywords: Cardiomyocytes, angiotensin, anti-hypertrophic, proliferation.

1. Introduction

Despite all the advances and advances that have been made in the area of cardiovascular disease, they are still a major public health problem and the leading cause of death

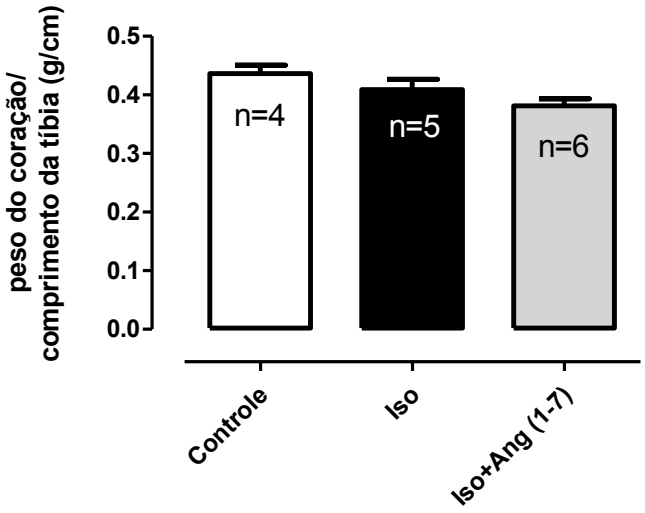
worldwide. A major limitation in this area comes from the inability of adult cardiomocytes to proliferate, which could recover the heart in the event of injury. From this perspective, in recent years a set of studies has demonstrated that there is a possibility of stimulating this proliferation of adult cardiomyomas and

Consequently cardiac regeneration. However, substances that have been demonstrated to exert this effect have great limitations in terms of their use as drugs. Data obtained by our research group indicate that Angiotensin- (1-7) (Ang- (1-7)), an endogenous peptide with a broad spectrum of cardioprotective effects, has already been included in pre- As an antihypertensive drug, has the potential to induce cardiomyocyte proliferation, thus bringing about a truly viable possibility for the stimulation of cardiac regeneration. Our central objective is to investigate whether Ang- (1-7) promotes cardiomyocyte proliferation and cardiac regeneration. For this, our experimental strategy encompasses techniques of histology, molecular biology, cell biology and fluorescence microscopy.

2. Results and Discussion

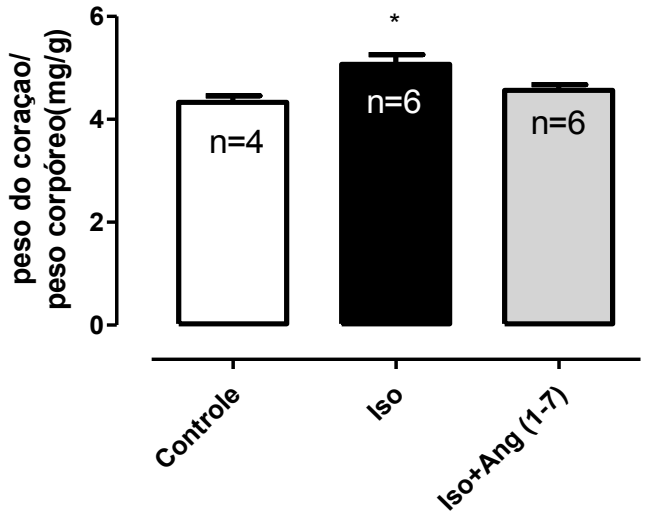
After the induction of cardiac stress by sioproterenol and treatment with Angiotensin- (1-7), we did the measurement of cardiac hypertrophy by means of the weight ratio of the heart by the weight of the tibia, we did not find differences between the groups, as expressed in the figure 1

Figure 1. Measurement of cardiac hypertrophy in adult wistar rats by weight ratio of Heart / tibia length (g / cm). (Iso = isoproterenol, Ang (1-7) = angiotensin 1-7) n = number of animals used.



When the ratio of the weight of the heart to the body weight was made, weHypertrophic response of sioproterenol, which was reversed by the use of angiotensin- (1-7), as expressed in figure 2.

Figure 2. Measurement of cardiac hypertrophy in adult wistar rats by weight ratio of Heart / body weight (mg / g). (Iso = isoproterenol, Ang (1-7) = angiotensin 1-7) n = number of animals used. * P <0.05 when compared to the control group.



Regarding the quantifications of the RNAs, they were made by means of spectrophotometry In Nanodrop 2000 (Thermo Scientific-USA) at wavelength A260 and A280 nm. To verify by real-time PCR technique the expression of genes involved in the process of cardiomyocyte proliferation. In this sense, we initially verified the quality of the RNA samples, by the A260 / A280 ratio, in which values greater than 1.8 indicate a high degree of purity. The values of each sample are presented in figure 3. Then we made the concentration of each sample, as shown in figure 4.

Figure 3. Quality analysis of purified RNAs by spectrophotometric analysis. Values ≥ 1.8 represent a high degree of purity. Iso = isoproterenol; Ang (1-7) = Angiotensin- (1-7)

Sample	A260/A280 nm
Control 1	2.09
Control 2	2.06
Control 3	2.06
Control 4	2.14
Iso 1	2.08
Iso 2	2.05
Iso 3	2.11
Iso 4	2.02
Iso+ Ang (1-7) 1	2.09
Iso+ Ang (1-7) 2	2.08
Iso+ Ang (1-7) 3	2.11
Iso + Ang (1-7) 4	2.13
Iso + Ang (1-7) 5	2.06
Iso+ Ang (1-7) 6	2.09

Figure 4. Dosage of purified RNAs by spectrophotometric analysis. Iso = isoproterenol; Ang (1-7) = Angiotensin- (1-7).

Sample	Concentração (µg/mL)
Controle 1	4.29
Controle 2	9.16
Controle 3	9.01
Controle 4	2.79
Iso 1	1.37
Iso 2	0.26
Iso 3	4.93
Iso 4	0.71
Iso + Ang (1-7) 1	3.53
Iso +Ang (1-7) 2	0.62
Iso + Ang (1-7) 3	2.84
Iso + Ang (1-7) 4	1.74
Iso + Ang (1-7) 5	1.74
Iso + Ang (1-7) 6	0.81

3. Materials and Methods

Dataset

This project was approved by the Department of Biochemistry and the Ethics Committee on the Use of Animals of the Biotechnology Center of the Federal University of Paraíba under the CEUA protocol 0204/13. Adult male Wistar rats, 250-300g and neonates 1-2 days old, will be used, from the Biotério Prof. Thomas George (CBiotec - UFPB). In order to obtain the cardiomyocytes, the standard procedure for

Enzymatic cleavage of the tissue as described by Guatimosim et al. (28). Briefly, through retrograde perfusion through the aortic artery, hearts will be digested using a collagenase-containing digestion solution and protease. The hearts will be perfused 10-15min with this solution, then the ventricular chambers will be removed, perforated and mechanical dissociation will be performed. After dissociation of ventricular cardiomyocytes, the cells will be maintained in DMEM (Dulbecco's Modified Eagles Medium) (Sigma) medium until use. For the in vitro experiments, with the objective of analyzing the effects of angiotensin- (1-7) on

Proliferation of adult cardiomyocytes, these cells, after dissociation, will be cultured in DMEM medium in a CO2 incubator (5%) at 37 ° C. Both adult and neonatal cardiomyocytes will be treated with Ang- (1-7) for at least 72 hours, with addition of the substances to cells every 12 hours. In order to generate a significant loss of ventricular mass, the

Induction of myocardial infarction using the technique of left descending coronary artery ligation. Briefly, the animals will be anesthetized and artificially ventilated. A thoracotomy will be performed, making an incision between the 3rd and 4th left ribs, to access the heart and the left descending coronary artery. A suture will be made in this artery in the region immediately anterior to its bifurcation, causing ischemia and Large left ventricular area.

Treatment of infarcted hearts with Ang- (1-7)

After the infarct generation procedure, the animals will be kept in recovery for two weeks,

after which the Ang- (1-7) administration process will be started. The administration of the compounds conjugated or not to the nanotubes will happen through injections

Intraperitoneal implants or through the implantation of osmotic mini-pumps, implanted subcutaneously (21)

Cellular proliferation analysis

Cellular proliferation analysis will be performed on isolated cardiomyocytes, decoration and heart slices. The BrdU (Bromodeoxyuridine) incorporation assay will be performed to check changes in DNA replication. In the in vitro experiments BrdU will be fired in the culture medium (30μM) for the last 48h of culture. In vivo experiments BrdU will be injected intraperitoneally (70 μmol / kg) in the last three days prior to the animal's sacrifice. The immunofluorescence technique will also be used for the observation of events of karyokinesis and cytokinesis, by labeling, with specific antibodies, key proteins in this process (eg α-tubulin, aurora-B kinase, phospho-histone 2).

For the performance of immunofluorescences, the cardiomyocytes will be fixed in paraformaldehyde (PFA) 4% in phosphate buffer (PBS) and permeabilized with 0.5% triton X-100. Anti-auroral B-kinase, anti-α-tubulin and anti-histone 2 antibodies will be used for identification of karyokinesis and cytokinesis, as well as Alexa-488 conjugated secondary antibodies (Molecular Probes). For analysis of the proliferation of cardiomyocytes directly in the heart, they will be fixed at 4% PFA in PBS. After fixing the hearts will be dehydrated and

included in paraffin, for later cut in microtome, in slices of 10μm. For visualization of the cells, the slides will be stained with hematoxylin and eosin (HE). For visualization of specific proteins involved in the proliferative process in cardiomyocytes, the immunofluorescence technique will be used in these histological sections of the heart. To verify cardiac function, echocardiography technique for the analysis of loss of cardiac function with infarction and changes promoted by Ang- (1) treatment will be used in control, infarct and infarct animals treated with Ang- (1-7) -7). The present project presents its feasibility of execution guaranteed, since the project coordinator masters all the necessary techniques for the execution of the same, guaranteeing the teaching of the same to the student executor of the project. In addition, the project presents a financial contribution from the Universal-CNPq process - # 475559 / 2012-6, and all the necessary equipment for the realization of the experiments are available and in full operation in the Laboratory of Cellular and Molecular Biotechnology, of which the project coordinator is an integral member, and the reagents necessary for the realization of the experiments are also already available for use.

4. Conclusions

The results indicate that Ang- (1-7) reverses the pathological remodeling generated by isoproterenol induced inducible cardiac stress. Additionally, we observed that the RNA samples underwent a satisfactory extraction and purification process, showing that the material is suitable for future analysis of gene expression. However, as it was not possible to identify the genes involved in the process of cardiomyocyte proliferation using the PCR technique, we have shown that further studies are needed to better understand the proliferation of cardiomyocytes. In addition, in vivo experiments to Angiotensin-(1-7) cardiac regeneration potential also need to be realized.

Acknowledgments

We are grateful to development agency CNPq.

Author Contributions

Main text paragraph.

Conflicts of Interest

The authors declare no conflict of interest.

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