Conditioned medium of sympathetic ganglia with addition of fibroblast growth factor 2 promotes plasticity of cardiomyocytes in culture

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**Abstract**

**Introduction:** The cardiac muscle cell, also called the cardiomyocyte, has specialized functions, playing a fundamental role in maintaining life, since it is responsible for the electrical and contractile activity of the heart. Like other specialized cells, it does not have an effective capacity for regeneration and its death or apoptosis is always an undesirable event, with sometimes catastrophic consequences, such as in Heart Failure (HF), which is known as the “final path” of heart diseases. Howe-
However, any cardiac disease in which cardiomyocyte apoptosis is implicated will develop, at some point in time, the HF syndrome. Evidence shows the influence of several substances as strong components responsible for inducing cardioprotection after tissue damage. **Objective:** In this perspective, this study aimed to analyze the plasticity of cardiomyocytes in the presence or absence of Fibroblast Growth Factor 2 (FGF-2) in the presence of sympathetic ganglia conditioned medium (MCGS). **Method:** For this, the behavior of the sympathetic ganglion in culture was analyzed, morphologically mapping the population and the ganglionar migratory profile, the growth and the morphology of the cardiomyocytes over 72 hours through the phase contrast microscopy, protocol number 006/15. **Results:** A statistically significant increase was observed both in the area and the cell perimeter in the groups treated with MCGS and MCGS with addition of FGF-2 (p = 0.0001) in relation to the control group. **Conclusion:** It is concluded, therefore, that there is an important plastic effect of MCGS, potentiated by FGF-2 in cultured cardiomyocytes.

**Introduction**

The cardiac muscle cell, also called the cardiomyocyte, has specialized functions and plays a fundamental role for the maintenance of life, since it is responsible for the electrical and contractile activity of the heart. Like other specialized cells, it does not have an effective capacity for regeneration and its death or apoptosis is always an undesirable event, with sometimes catastrophic consequences, as occurs in Heart Failure (CI) (Braunwald, 2013).

HF is known as the “final pathway” of heart disease. Any cardiac disease in which cardiomyocyte apoptosis is implicated will develop, at some point in time, the HF syndrome. Energy inefficiency or oxidative processes deleterious to cell survival plays an important role in the development of cardiomyocyte death (Braunwald, 2008). Events such as Coronary Artery Disease, which can result in complications such as acute myocardial infarction is an im-
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HF is a complex and comprehensive clinical syndrome, sometimes with imprecise limits on its definition, given the heterogeneity of heart diseases that may lead to the development of HF (Krum & Abraham, 2009). It affects approximately 38 million people worldwide, representing a global health problem, being more frequent in populations over 65 years (Braunwald, 2013).

However, the clinical condition observed in the HF syndrome is only a result of multiple molecular, cellular and neuroadaptive dysfunctions, culminating in the dysfunction of the intercellular functioning of the cardiomyocyte and cardiac fibroblasts, with loss of function and consequent cardiac organ failure (Braunwald, 2013; Bang et al., 2015; Neubauer, 2007; Moore-Morris et al., 2016).

Nevertheless, in situations of cardiac injury, the neuronal response due to myocardial injury causes direct consequences in cardiac functioning, leading to a reduction in cardiac output, causing reduction of vascular filling, a situation detectable by arterial baroreceptors (Fukuda et al., 2015). Initially, as an adaptive attempt in a situation of abnormality, through baroreceptors, there is afferent activation of the neuroendocrine system, with increased sympathetic tone and reduction of the parasympathetic tone, promoting increased cardiac output, elevated heart rate and peripheral vascular resistance (Kember, Armour & Zamir, 2013).

Sympathetic ganglia, as well as most peripheral autonomic nervous system (ANS) cells, originate from the symmetrical lateral portions of the neural plate (Boron & Boulpaep, 2015). The sympathetic, preganglionic neuronal cell bodies are located in the gray matter of the lateral horns of the spinal cord. The axons of the
preganglionic neurons exit the spinal cord by the ventral roots of the spinal nerves (VanPutte, Regan & Russo, 2016; Gibbins & Morris, 2006; Kawashima, 2005).

Cardiac sympathetic innervation, almost entirely, arises from such ganglia, although there is a description of postganglionic cell bodies present in the myocardium itself. These neurons are responsible for modulating cardiac functions vital to its functioning, such as chronotropism, dromotropism, lusitropism and inotropism (Coote & Chauhan, 2016).

Most sympathetic postganglionic axons responsible for innervation of the heart originate from cell bodies in the stellate ganglion (Pardini, Lund & Schmid, 1989), or caudal cervical ganglion. Upon reaching the heart, the sympathetic nerves branch out abundantly to innervate the 3 cardiac tracts (pericardium, myocardium and endocardium) (Baptista & Kirby, 1997).

In situations of cardiac injury, mechanisms such as apoptosis and autophagy become present. While apoptosis represents an active process in the evolution of cardiac dysfunction, autophagy seems to exert a double and antagonistic function (Nishida & Otsu, 2016). Initially, autophagy protects the heart, destroying cardiomyocytes with mitochondrial and structural dysfunctions, presenting as an adaptive mechanism in situations such as HF. However, it can also develop deleterious potential, such as apoptosis, since the abundant destruction of cardiomyocytes will make the myocardium even more insufficient, as well as disseminating, through autophagic vacuoles, various cytotoxic substances (Nishida & Otsu, 2016).

There is, therefore, an incessant search for the emergence or improvement of techniques capable of protecting cardiomyocytes from inflammatory and oxidative processes that would lead to cell death. This search has led to several directions, such as the transplantation of skeletal muscle cells to the heart, in order to assume a similar function to those performed by the cardiomyocyte; transplantation of stem cells into fibrous cardiac areas; application of trophic factors, improving
cellular vascularization, and the administration of antioxidant and anti-inflammatory factors, which could reduce the rates of cellular apoptosis in certain conditions unfavorable to cardiomyocyte functioning (Braunwald, 2013).

Fibroblast Growth Factors (FGFs) present a prominent role as a perspective for the treatment of heart diseases. They are polypeptides with diverse biological functions, from tissue development to metabolic control, being responsible for the proliferation, migration, differentiation and survival of cells expressing FGF receptors (Boyd & Gordon, 2003; Hotta et al., 2008; Eswarakumar, Lax & Schlessinger, 2005). In the heart, they exert influence from development and differentiation, to the mechanism of autophagy and cardiomyocyte apoptosis (Rosenblatt-Velin et al., 2005).

Thus, the effect of the substances produced by the sympathetic ganglia and the actions of FGFs on the functioning of the cardiomyocyte emerges as an important field of research for the development of adjuvant and effective therapies in cases of cardiac injury.

Materials and Methods

Experimental draw

Four animals (Wistar rats - Rattus novergicus), aged 40 to 50 days and weighing approximately 200 grams, were used, which were kept on a ventilated shelf with bacteriological flow for one week before being used. In a collective cage (30 x 16 x 19 cm), with 2 animals per cage and average temperature of 22 ± 2°C, fed with standard feed and water supplied ad libitum. The project complied with the norms for conducting research on animals with all procedures, and was approved by the Commission of Ethics in Animal Experimentation (CEEA) of the State University of Rio Grande do Norte (UERN), receiving protocol number 006/15.

Extraction and culture of sympathetic ganglia and cardiac ventricular myocardial cells

Within the laminar flow, culture plates, P60 were prepared with 5 ml of Leibovitz-15 medium (L-15: GIBCO Invitrogen Corpo-
ration). In the animals submitted to the extraction of the sympathetic ganglia (2 animals), euthanasia was performed with an excessive dose of Isoflurane (Halotano®) and, afterwards, tricotomy performed in the anterior and abdominal thoracic regions, performing local asepsis with alcohol at 70%. The surgical approach was performed in the medial region of the anterior face of the trunk, removing the muscular and bony planes, removing the viscera blocks from the trunk and abdomen, resulting in the paravertebral chain (sympathetic trunk) of the sympathetic ganglia.

The sympathetic ganglia were excised and placed in P60 plates with L-15 medium, under aseptic surgical technique with the aid of microinstruments (scissors, tweezers, retractors). All excess tissue (muscle, fat, and blood vessels) was removed under magnification by SZ61 (Olympus®) stereoscopic magnifying glass.

The dissected sympathetic ganglion paravertebral strand was segmented into isolated ganglia within the laminar flow, and these were plated on P30 plates with 2mL Dulbecco’s modified Eagle’s medium medium supplemented with 10% fetal bovine serum and 10μg / mL of gentamicin, the so-called D-10 medium, all of which were obtained from Cultilab®. Four sympathetic ganglia (stellar - cervicothoracic and caudal ganglia), both bilateral, were added to each P30 plaque.

The excess of the medium was removed so that the ganglia did not float, nor even submerged in the middle. As time progressed the conditioned medium from the migration and lymph node activity was collected after 2 weeks of migration, placed in eppendorfs of 1.5mL and packed in a freezer -80ºc, which was used to treat the cardiomyocytes in culture, addition or not FGF-2.

Left ventricular regions were removed from the heart of 2 Wistar rats and then subjected to extraction of myocardial fragments. For this, the protocol of Chlopcikova et al., (2001) (67) was used. Rats (2 animals) were sacrificed with lethal dose of isoflurane anesthetic (Halotano®) and performed thoracotomy for removal of hearts. Next, the atria...
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and ventricles were separated, the atria being disregarded and the ventricles immersed in a 15 mL falcon tube containing 4 mL of L-15 medium. Inside the laminar flow, the process of cleaning and ventricular isolation on P60 plate with L-15 was performed, using scissors, tweezers and a small spatula, resulting in the collection of myocardial fragments.

After obtaining the myocardial fragments, they were deposited in P30 plates with 2 ml of D-10 and subjected to successive cycles of enzymatic digestion with 5 ml of 1mg/ml solution of trypsin (supplied by Call Fisher) overnight and mechanical digestion with repetitive pipetting.

The sample was centrifuged at 1500 rpm for 5 minutes, 3 times for the purpose of recovering the cell decanter. The cells obtained with enzymatic and mechanical digestion cycles were then resuspended in 5 mL of D-10. Then, seeded in Petri dishes of P30 and incubated for forty minutes in an incubator with 5% CO2 atmosphere at 37 °C.

Cardiomyocytes were normally separated from the mesenchymal cells (fibroblasts) by time of differential adhesion to the P30 plaque. Mesenchymal cells adhere more rapidly to plaque in relation to cardiomyocytes (Blondel, Roijen & Cheneval, 1971; Chlopcikova, Psotova & Miketova, 2001). After the incubation period of 40 minutes and in suspension, cardiomyocytes were collected by aspiration of the culture medium and then counted in Neubauer’s chamber and the same cell population was defined for each plate, thus the populations of the cardiomyocytes used were homogeneous.

P30 were prepared with 2 ml of fetal bovine serum, removed and discarded after 30 minutes. Next, 2 mL of D-10 was added, then the newly extracted cells were dripped and kept in a humid oven at 370C with 5% CO2 and 95% air. Phase contrast inverted light microscopy was used to observe cell adhesion at the bottom of the wells.

Subcultures of cardiomyocytes in experimental groups

The cells were deposited in 24P30 with 2 ml of D-10 medium, 6 plates for each group and observed in three time periods: 24, 48 and 72 hours. With this procedure it was
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possible to evaluate the adherence and plasticity/trophism of the cardiomyocytes in the following groups: Group 1 (G1): Cardiomyocyte + D-10 medium; Group 2 (G2): Cardiomyocyte + medium D-10 + MCGS; Group 3 (G3): Cardiomyocyte + medium D-10 + MCGS + FGF-2 and Group 4 (G4): Cardiomyocyte + D-10 + FGF-2 medium.

In groups 2 and 3, 0.2 ml of D-10 medium was removed and 0.2 ml of MCGS medium was collected, which was collected from the medium where the sympathetic ganglia were plated.

**Phase Contrast Microscopy**

For cellular observation, a MCK CKX41 (Olympus®) with Moticam 3.0 digital camera (Motic®) was used.

For cellular morphometric analysis, the images were captured in 9 fields initially demarcated, not overlapping in the 20x magnification, in this way, microphotographs of the 4 groups were made in the 3 time periods.

**Data analysis**

Two independent and blind investigators analyzed the cell morphometry, thus proceeding with counting the cells in a calibrated prediction system (Kappa = 0.94) by absolute number field, by measuring the perimeter (μm) and area (μm²) of each cell in not overlapping visual fields at 20x magnification. Motic Images Plus 2.0 (Motic®) and Image-J software were used for morphometric observation, as well as Adobe Photoshop CS6.0 (Adobe®) software for minimal correction of brightness and contrast of photomicrographs.

The survey database was built on the software platform, with subsequent checking for consistency of typing. After the final structuring of the database, a descriptive analysis of all data (area and cell perimeter) was performed initially.

Cellular morphology data over the 72 hours were compared statistically through analysis of variance (51) with Fisher and Bonferone test powders considering a significant difference when p <0.05.

**Results**
In cultures with sympathetic ganglia (24hs) the migration of a large cell population (Figure 1A) was observed, these cells being identified as satellite cells (arrowhead) (Figure 1B).

**Figure 1**: The figures illustrate the cellular migration of the sympathetic ganglion (A and B, increases of 10 and 40x, respectively) in the first 24 hours, and the morphological pattern of the satellite cells pointed by the arrow head increased by 40x). In the next 48 hours we observed the migration of a second cellular profile (fibroblasts) of the sympathetic ganglion (C in a 10x magnification) and the morphological pattern of the fibroblasts (arrow) and the satellite cells (arrowhead) in D in the 20x magnification. After 72 hours a third cellular profile (neurons) of the sympathetic ganglion was visualized. Neurons are pointed by the arrow and the satellite cells by the arrowhead in E at 10x magnification and F at 40x magnification after 72hs. Scale 100μm.

In the period of 48hs in culture the migration of the cells with fibroblastic profile of the sympathetic ganglia (arrow) was observed. We observed the sympathetic ganglion
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and the cell migration profile, fibroblasts (arrow) and satellite cells (arrowhead) (Figure 1C and 1D).

After 72 hours the migration of sympathetic ganglia neurons was observed. We observed the sympathetic ganglion and the cell migration profile (Figure 1E). Neurons (arrow) and satellite cells (arrowhead) (Figure 1F).

Myocardial-derived cell cultures, when cultured in MCGS and FGF-2 (Figure 2G-24hs, H-48hs and I-72hs), showed more visible changes when compared to cultures cultured only in D-10 medium (Figure 2A-24hs, B-48hs and C-72hs) or only with FGF-2 (Figure 2J-24hs, L-48hs and M-72hs), especially with (arrow) of cardiomyocyte morphology, resulting in a plastic effect (Figure 2).

Considering the measurement of the cellular perimeter of the cardiomyocytes as a morphometric parameter, it was observed that over the three days of observation of groups 1, 2, 3 and 4 (D-10, MCGS, MCGS + FGF-2 and FGF-2, respectively) the cells increased their perimeter. At day 1 (24 hours), the mean perimeter of the cells of groups 2, 3 and 4 were higher than those of group 1, showing the same value of (*p=0,0001) between them. In the comparative analysis between groups 2, 3 and 4, we observed that group 3 presented a more extensive perimeter when compared to groups 2 and 4 (***p=0,0001). In addition, it was observed that group 2 showed a larger perimeter when compared to group 1 (*p=0,016) (Figure 3A).

**Figure 3:** Graphical representation shows the mean and standard error of the mean perimeter (A) and area (B) levels of the cardiomyocytes treated with D-10, MCGS, MCGS + FGF-2 or FGF-
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2. The analyzes were conducted daily for 3 days, followed by ANOVA of multiple comparisons with Tukey and Bonferroni tests for comparison between the groups in the analysis periods.

At day 2 (48 hours) and 3 (72 hours) of analysis, the mean perimeter of the cells of groups 2, 3 and 4 remained higher than those of group 1, as presented in 24 hours (**p=0.0001). In the comparative analysis between groups 2, 3 and 4 we observed that group 3 presented a larger perimeter when compared to groups 2 and 4 (**p=0.0001). In addition, we observed that group 2 increased its perimeter when compared to group 1, thus increasing its significance level from a value of (*p=0.016) presented in 24 hours to a value of (**p=0.0001) in 48 hours, if holding at 72 hours (Figure 3A).

Considering the measurement of the cellular area of the cardiomyocytes as a morphometric parameter, it was observed that during the three days of observation of groups 1, 2, 3 and 4 the cells increased their area. At day 1 (24 hours) and day 2 (48 hours), the area of the cells of groups 2, 3 and 4 were higher than those of group 1, presenting the same value of (**p=0.0001). In the comparative analysis between groups 2, 3 and 4 we observed that group 3 had a larger area when compared to groups 2 and 4 (**p=0.0001). In addition, it was observed that group 2 showed a larger area when compared to group 1 (**p=0.0001) (Figure 3B).

At day 3 (72 hours) of analysis, the cells area of groups 2, 3 and 4 remained higher than those of group 1, as presented in 24 and 48 hours of analysis (**p=0.0001). In the comparative analysis between groups 2, 3 and 4, we also observed that group 3 had a larger area when compared to groups 2 and 4 (**p=0.0001). In addition, we observed that group 4 decreased its cell area when compared to group 1, thus reducing its level of significance of a value of (**p=0.0001) presented in 24 and 48 hours to a value of (*p=0.018) (Figure 3B).

In the analysis of the time of observation regarding the cellular perimeter of the cardiomyocytes,
it was observed that throughout the three days of observation of the group 1 the cells increased their perimeter (24 for 48 hours **p=0.001, 24 and 48 for 72 hours ***p=0.0001) as shown in figure 4A.

**Figure 4**: Graph showing the mean and standard error of the mean perimeter and cell area of cardiomyocytes treated with D-10, MCGS, MCGS + FGF-2 or FGF-2. The analyzes were conducted daily for 3 days, followed by ANOVA of multiple comparisons with Tukey and Bonferroni tests for comparison between the groups in the analysis periods.

Regarding the observation time for the cardiomyocyte cell area as a morphometric parameter, it was observed that throughout the three days of observation of the group 1 the cells increased their area presenting e value ***p=0.0001 in the three periods as shown in figure 4B.

In the analysis of the time of observation regarding the perimeter and cellular area of the cardiomyocytes, it was observed that throughout the three days of observation of the group 2 that the cells increased their area presenting value of ***p=0.0001 in relation to the comparison between the periods Figure 4C, D, E, and F.

In the analysis of the observation time for the cardiomyocyte cell perimeter, it was observed that throughout the three days of observation of group 4 that the cells increased their perimeter (24 for 48 hours **p=0.002, 24 and 48 hours for 72 hours ***p=0.0001) as shown in figure 4G.

Regarding the observation time for the cardiomyocyte cell area as a morphometric parameter, it was observed that throughout the three days of observation of
group 4, the cells increased their area (***p=0.0001) in relation to the comparison between the periods as shown in figure 4H.

Discussion

Currently, several studies point to important effects of FGF-2 on the cardiomyocyte, especially on the prevention of cell death (Kardami et al., 2007). Such effects may be determinant since the onset of alterations in cardiomyocyte relaxation, leading to ventricular diastolic dysfunction until the inhibition of cellular oxidation mechanisms and mitochondrial dysfunction (Chen et al., 2016).

The interaction of FGF-2 with the cardiomyocyte develops as a looping action, since the cardiomyocyte itself is capable of producing FGF-2, which will act on itself since embryogenesis, in addition to participating in angiogenesis (Krejci, Pesevski, Nanka & Sedmera, 2016). Evidence points to an apparently paradoxical function of FGF-2 and other FGF molecules in cardiogenesis itself, determining the differentiation of precursor cells in cardiomyocytes at early stages of development but preventing premature differentiation at later stages through action in the determination and control of cellular apoptosis (Zhang et al., 2012). Such effects have led to the search for pathways for the differentiation of fibroblasts into cardiomyocytes, through the adjuvant effects of FGF-2 (Yamakawa et al., 2015).

Experimental studies have explored the important relationship between FGF-2 and the development of cardiac muscle cells. Direct reprogramming of fibroblasts, for example, to cells with cardiomyocyte-like functions became an inefficient process, but the use of FGF-2, in addition to the action of the GATA-4 Transcription Factor, proved to be an efficient process, opening new frontiers for cellular reprogramming and development of therapies against heart disease (Yamakawa et al., 2015). In addition, the addition of low doses of the “hi-FGF-2” form in cardiomyocyte culture has positive effects on the mitogenic potential of such cells, if performed at early culture times, but promote inhibition of cell
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proliferation and death if administration of the molecule is done in high doses and late periods of the culture (Hirst et al., 2003). The activity of FGF-2 was also demonstrated in a study of the regulation of fetal myocardial proliferation, in which the increase of DNA synthesis was evidenced through the action, among other factors, of this growth factor (Armstrong & Armstrong, 2009).

In studies of repair or recovery of necrotic cardiac areas, similar to that observed in cases of infarction, FGF-2 also demonstrates important function. In a study of the regeneration capacity of the cardiac muscle cell, it has been shown that FGF-2 can be used in vivo to allow both migration and differentiation of resident ‘precursors’ of cardiac cells (Rosenblatt-Velin et al., 2005). The demonstration of the increased survival rate of cryoglobulin-induced implanted necrotic bone marrow stem cells when treated with FGF-2 (Song et al., 2005) is important evidence of how promising the use of FGF-2 in the treatment of heart lesions considered irreversible.

In the present study, we observed the positive plastic effect when cultivating cardiomyocytes with the addition of FGF-2, especially in the group with the addition of conditioned medium of paravertebral sympathetic ganglia. We observed that the addition of the conditioned medium in the culture of cardiomyocytes favors its development. In this way the cells have a more organized structural support to carry out their functions. In addition, we observed that the effect of the conditioned medium on paravertebral sympathetic ganglia is enhanced by the addition of FGF-2, thus showing a synergistic effect on the morphometric development of cardiomyocytes. This effect is of paramount importance in cell recovery in the process of cell death. Thus, the conditioned medium of paravertebral sympathetic ganglia has components in its extracellular matrix that are crucial for the functionality of the cardiomyocyte. Such findings are corroborated by several studies that point to a precise and intrinsic relationship between the sympathetic system and the heart, in which there is a “neuro-cardiac” junction, where
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sympathetic neurotransmission would occur in a pathway similar to neuronal synapses (Conforti, Tohse & Sperelakis, 1991; Zaglia & Mongillo, 2017), besides the presence of postganglionic neurons projecting to various areas of the heart (Kucera & Hrabovska, 2015). In addition, the effects of sympathetic and parasympathetic neurons play an important role in cardiomyocyte culture (Oiwa et al., 2016).

In a study of the combined culture of cardiomyocytes and autonomic neurons from the star ganglion, the anatomical interaction is evidenced by numerous axonal projections on the cardiomyocyte (Landis, 1976), as well as functional between the two cells (Horackova, 1993). Direct contact between neurons and cardiomyocytes in culture can lead to increased functional expression of calcium channels, which are essential for cardiomyocyte activity and survival (Ogawa et al., 1992). The interrelation between the sympathetic nervous system and the cardiomyocyte is also evidenced by findings suggesting that the sympathetic system, through its effects on the cardiomyocyte, is one of the important factors responsible for the proliferation and hypertrophy of these cells, thus contributing to the development of neural control in the cardiomyocyte adult heart (Kreipke & Birren, 2015). It is also attributed to the sympathetic neurons the stimulation to the contraction of isolated cardiomyocytes in culture, as well as to the development of the cardiac function itself (Lloyd & Marvin, 1990).

However, the interaction between cardiomyocytes and sympathetic neurons extends far beyond the neuro-cardiac junctions. Neurotrophins, initially described as function and neuronal development, also play a role in the cardiovascular domain, contributing to the homeostasis of this system (Pius-Sadowska & Machalinski, 2017). Therefore, they play an important role in the modulation of synaptic properties of the autonomic neurons, axonal projection and development, neural and vascular networks formation, smooth muscle migration and control of endothelial cell and cardiomyocyte apoptosis (Pius-Sadowska
Conditioned medium of sympathetic ganglia with addition of fibroblast growth factor 2 promotes plasticity of cardiomyocytes in culture & Machalinski, 2017; Osadchii, 2015).

The evidence of neuro-cardiac interrelation becomes more consistent when neurons and cardiomyocytes demonstrate that neurons exert an important influence on the expression of beta-adrenergic cardiomyocyte phenotype, which are sensitive to diseases mediated by adenosine response (cAMP), an important molecule in the determination of the cardiomyocyte response to the sympathetic system. Neuronal activity may therefore be an important determinant in the expression of cardiomyocytes with potential for the development of hypertensive disease (Larsen, Lefkimmiatis & Paterson, 2016).

From the neuro-cardiac interaction, there are indications of advances, although still in development, of therapy based on stem cell culture, using substances produced by neurons, such as neuronal growth factor, as well as its activity in the development of the cardiomyocyte (Coskun & Lombardo, 2016). The possibility of reaching gene therapies using stem cell cultures to derive sympathetic neurons with potential to form interaction, both anatomically and functionally, has already been demonstrated (Oh et al., 2016).

In this context, perspectives of molecular treatment of HF appear. The demonstration that enzymes, such as the sarcoendoplasmic reticulum calcium ATPase 2a, associated with the development of HF in cytomegalovirus infections, promote sympathetic neuronal hyperactivation, with consequent greater sympathetic excitation in the cardiomyocyte (Shanks et al., 2017), indicates the importance of understanding this interaction. Although it is not a recent knowledge, the action of adenosine triphosphate (ATP), released by neurons, together with noradrenaline, as co-transmitters also plays a fundamental role in intercellular communication and is also an important perspective in the understanding not only of heart diseases, but also vascular diseases (Burnstock, 2017).

Also, no less important is the recognition of the potential for development of embryonic stem cell therapy. The generation of
cardiomyocytes, from embryonic stem cells, depends on the expression of transcription factors such as GATA-4, which is induced by fibroblast growth factors (Sachini-dis et al., 2002).

Thus, the search for the extensive and adequate understanding of the interaction between the actions of neuronal factors and FGFs in the cardiomyocyte becomes a promising field of research in the future treatment of cardiomyopathies.

**Conclusion**

Cardiomyocytes showed a typical morphology, rapid expansion and trophic effect, especially in the group that was exposed to the paravertebral sympathetic ganglion conditioned medium with addition of FGF-2. This plastic effect is due to properties found in the conditioned medium, being potentiated by the addition of FGF-2, as this synergistic effect allowed a rich, nutritious environment with great electrical potential, ideal for the survival and structural and functional development of the cardiomyocyte.

The present study improves the knowledge about the plasticity of cardiomyocytes and facilitates the understanding in the search for better techniques with cellular therapy in the use of cardiac injuries. In addition, the conditioned medium opens up an option of producing a synthetic substance based on its chemical constitution.
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