Peroxisome Proliferator-Activated Receptors (PPAR) down-regulate the expression of pro-inflammatory molecules in an experimental model of myocardial infarction

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Peroxisome Proliferator-Activated Receptors (PPAR) down-regulate the expression of pro-inflammatory molecules in an experimental model of myocardial infarction

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Abstract

Myocardial infarction (MI) has been associated with an inflammatory response and a rise in TNF α , interleukin (IL)-1 β , and IL-6. Peroxisome proliferator-activated receptors (PPARs) promote a decreased expression of inflammatory molecules. We aimed to study if PPAR stimulation by clofibrate decreases inflammation and reduces infarct size in rats with MI. Male Wistar rats were randomized into three groups: control, MI + vehicle, and MI + clofibrate (100 mg/Kg). Treatment was administered three consecutive days, previous to 2 h of MI. MI induced an increase in protein expression, mRNA content, and enzymatic activity of inducible nitric oxide synthase (iNOS). Additionally, MI incited an increased expression of matrix metalloproteinase (MMP)-2 and -9, intercellular adhesion molecule (ICAM)-1, and IL-6. MI also elevated the nuclear content of nuclear factor- κ B (NF- κ B) and decreased I κ B, both in myocyte nuclei and cytosol. Clofibrate treatment prevented MI-induced changes in iNOS, MMP-2 and -9, ICAM, IL-6, NF- κ B, and I κ B. Infarct size was smaller in clofibrate-treated rats compared to MI-vehicle animals. *In silico* analysis exhibited 3 motifs shared by genes from renin angiotensin system, PPAR- α , iNOS, MMP-2 and -9, ICAM-1 and VCAM suggesting a cross regulation. In conclusion, PPAR α -stimulation prevents overexpression of pro-inflammatory molecules and preserves viability in an experimental model of acute MI.

Keywords: PPAR, iNOS, fibrates, myocardial infarction, interleukins, cell adhesion molecules, matrix metalloproteinases, NF- κ B, I κ B.

Introduction

Acute myocardial infarction (AMI) has been associated with an immediate inflammatory response accompanied by a gain in cytokine production, e.g. tumor necrosis factor- α (TNF α), interleukin (IL)-1 β and IL-6 (Mann 2003). The extension of ischemic area is a determinant factor for cytokine cytotoxic potential. Accordingly, a small infarcted area exhibits a temporary rise of cytokine production, while a large infarcted area is able to overproduce sustained amounts of cytokines leading to chronic heart remodeling (Ono et al. 1998). Indeed, in the infarcted area, cytokines exert a stimulating effect on the production and activity of matrix metalloproteinase (MMPs)-2 and -9, while in the non-infarcted area, the production of collagen increases (Deten et al. 2002). At the functional level, cytokines impair left ventricular functioning and myocyte contractility through a reduction of cytosolic Ca²⁺ and an altered function of sarcoplasmic reticulum (Yokoyama et al. 1993).

Stimulation of peroxisome proliferator-activated receptors (PPARs) has been reported to decrease the expression of inflammatory mediators. Evidence was obtained from PPAR α -/- mice, who exhibited a more intense and longer inflammatory process compared with wild type mice (Devchand et al. 1996). It has also been reported that fibrate-treated dyslipidemic patients lower their production of cytokines, such as TNF- α , interferon gamma (IFN- γ), IL-6, and c-reactive protein (CRP)(Li et al. 2006). The anti-inflammatory molecular mechanism triggered by PPAR α stimulation includes the interaction (Chinetti et al. 1998) of PPAR α and repressor factors such as c-Jun and nuclear factor- κ B (NF- κ B) subunit p65 (Delerive et al. 1999).

The activity of NF- κ B is controlled by the interaction with its inhibitor, I κ B, which binds to cytosolic NF- κ B dimers rendering them inactive. In primary cultures of smooth muscle cells and hepatocytes, active PPARs also partake in inducing the expression of I κ B α at the mRNA and protein level (Delerive et al. 2000). The increase in I κ B production occurs in the nuclei and blunts the binding of NF- κ B to DNA, but it shows no effect on NF- κ B translocation to the nuclei. This suggests that PPAR α agonists are potential pharmacological agents that decrease inflammation. However, their participation in myocardial ischemic events has not been fully elucidated. Therefore, the aim of this protocol was to study if clofibrate-induced PPAR α stimulation in an experimental model of myocardial infarction (MI) prevents the associated inflammation and limits the extension of the infarcted area.

Material and methods

Male Wistar rats (250-300 g), bred and raised in our facilities (Instituto Nacional de Cardiología Ignacio Chávez), were randomly assigned to one of three different groups (n=6): Control, MI + vehicle (MI-V) (i.p.) or MI + clofibrate (MI-C) [100 mg/Kg, (i.p.)]. Rats were administered either vehicle (groups 1 and 2) or clofibrate (group 3) daily for 3 days before sham surgery or MI.

All animal procedures were conducted in accordance with Federal Regulations concerning Animal Experimentation and Care (Ministry of Agriculture, SAGARPA, NOM-062-ZOO-1999, Mexico). Animal protocol experimentation was approved by the Ethical

Committee of our Institution and conducted according to the Guidelines for Care and Use of Experimental Animals.

Myocardial ischemia

On the third day of treatment, rats were anaesthetized (ketamine/xylazine, 100/20 mg/Kg i.m.), artificially ventilated (70 strokes/min, 8-10 mL/Kg). Also, they had the heart exteriorized, and left anterior coronary descendent artery (LAD) occluded by means of a 7-0 silk. The heart was repositioned into the chest and sides re-approximated with 3-0 silk (Yue et al. 2003). Two hours later, the animals were sacrificed and had their heart removed. After this, blood samples were taken for further analysis. Control rats underwent the same procedure, except for LAD ligation. Samples were stored at -70 °C for further analysis. (Ibarra-Lara et al. 2012)

Infarct size

Hearts from control and MI (vehicle- and clofibrate-treated) rats were retrogradely perfused through abdominal aorta with heparinized PBS (0.1% pH=7.2-7.4) and Evans blue dye (2%). The hearts were frozen sectioned and were incubated with 2,3,5-triphenyl tetrazolium chloride [TTC (1%)/PBS (20mM pH 7.4)] at 37 °C (Yue et al. 2003).

mRNA inducible nitric oxide synthase (iNOS) expression

Ischemic areas from MI-V and MI-C rodents, and corresponding areas from control rats were obtained under sterile conditions, and processed according to González-Barrios. A 395-pb iNOS fragment was amplified; the sense primer was 5'-CCA CAA TAG TAC ATT ACT ACT TGG-3' and the antisense primer was 5'-AGG AGG TGT TCA TCA GCG TGC TCC ACG-3'. β -actin was used as a housekeeping gene (Gonzalez-Barrios et al. 2002).

Nitric oxide synthase activity

Enzymatic activity was analyzed as previously described by our group (Sanchez-Mendoza et al. 1998). Briefly, ischemic tissue or the corresponding area from control left ventricle was homogenized with a proteolytic enzyme inhibitor cocktail containing leupeptin 10 μ g/mL and aprotinin 20 μ g/mL. The following mixture was added to the volume of homogenate containing 500 μ g of protein: NAD(P)H 1 mM, calmodulin 100 nM, BH_4 30 μ M, CaCl_2 2.5 mM, L-arginine-HCl 10 μ M, and [^3H]-L-arginine 0.2 μ Ci (66 Ci/mmol). The final volume was evenly brought to 100 μ L with homogenization buffer and incubated at 37 °C for 30 min. The enzymatic reaction was stopped by the addition of 1 mL of stop buffer. Considering that nitric oxide (NO) and L-citrulline are co-products of NOS, the activity of NOS is expressed as ng of [^3H]-L-citrulline / 500 μ g protein / 30 min.

Determination of IL-6

Serum content of IL-6 was determined by ELISA, following the commercial kit manufacturer's instructions (DRG International, Marburg, Germany).

Nuclear and cytoplasmic extraction

Using liquid nitrogen, 40 mg of ischemic left ventricle was homogenized and samples were further processed following NE-PER[®] (*Nuclear and Cytoplasmic Extraction Reagents*, Thermo Scientific, Rockford, IL USA) manufacturer's instructions.

Evaluation of protein expression of ICAM-1, VCAM-1, MMP-2, MMP-9, NF- κ B, and I κ B.

Protein expression was evaluated by Western blot as previously described (Ibarra-Lara et al. 2012). Briefly, the volume containing 50 μ g of protein from homogenized left ventricular ischemic area or the corresponding area from control rats was electrophoretically separated to identify, by means of primary and secondary antibodies, the following proteins: NF- κ B (p65, 1:250), I κ B α (1:100), MMP-2 (1:300), MMP-9 (1:300), intercellular adhesion molecule-1 (ICAM-1, 1:250), vascular cell adhesion molecule (VCAM-1, 1:250), and lamin A as a loading control. A chemiluminescence kit (Millipore, Billerica, MA, USA) was employed to develop the bands.

Evaluation of ICAM-1, MMP-2, and -9.

Frozen tissue sections from ischemic and non-ischemic left ventricles were cut, fixed blocked (PBS/Azide 0.02%/BSA 1%) and incubated with primary antibodies for ICAM-1, MMP-2 and -9 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and secondary antibodies [goat anti-rabbit FITC (Jackson ImmunoResearch Laboratories Inc. West Grove, PA) and rabbit anti-goat FITC (Santa Cruz Biotechnology, Inc., Santa Cruz, CA)]. Nuclei in tissue sections were counterstained with DAPI (Vectashield, Vector laboratories, Inc) and analyzed by confocal microscopy (FV1000 Olympus Corporation, Tokyo, Japan). Wavelength for emission and excitement were set according to FITC and DAPI technical specifications. Images were captured and processed using FV10-ASW software (Olympus Corporation, Tokyo, Japan).

Palmitoyl CoA oxidase assay

In order to evaluate if clofibrate (100 mg/Kg) was appropriate to activate PPAR α in the heart, we measured the activity of peroxisome palmitoyl CoA oxidase, following the method previously described (Ibarra-Lara et al. 2010). Ischemic myocardium from control, MI-V, and MI-C rats were homogenized (1:3 w/v) in 0.25 M sucrose, 1 mM EDTA, and 0.1% ethanol. Samples containing 500 μ g of protein were incubated at 37°C for 30 min, with the reaction mix containing 60 mM Tris-HCl, 35 μ M palmitoyl CoA, 50 μ M FAD, 1 μ M scopoletin peroxidase (3 units), 0.6 mg bovine serum albumin, and triton X-100 (0.01%) to a final volume of 1 mL. The reaction was stopped by the addition of 4 mL of 0.1 M borate buffer (pH 10). Fluorescence was measured at 470 nm emission and 395 nm excitation with a Varian Cary Eclipse Fluorescence Spectrophotometer. Data is expressed as nmol scopoletin per mg of protein in 30 min.

***In silico* analysis**

Nucleotidic sequences from 5'-untranslated region (5'UTR) genes were obtained from Ensembl (NCBI) (http://www.ensembl.org/Rattus_novergicus/Gene/) to build a FASTA file. This file was further analyzed with Multiple Em for Motif Elicitation (MEME) program (<http://meme.sdsc.edu/meme/intro.html>) to identify motifs shared between them, which might possess characteristics, such as transcriptional regulatory binding sites, DNA binding sites, or protein interaction domains. Software predetermined conditions were employed for the study (Perera et al. 2006) (Sanchez-Aguilar et al. 2013). Later, the motifs obtained were searched on TESS database (www.ebil.upenn.edu/cgi-bin/tess/tess) to identify if these motifs had been reported as transcription factors, DNA binding sites, or protein interaction domains. Additionally, we quantified the percentage of nucleotides in the motifs.

Data analysis

The results are expressed as the media $\pm$ standard error of the mean. Statistical differences were calculated by an analysis of variance (ANOVA) followed by a Tukey-test. A p value <0.05 was considered significantly different.

Results

Palmitoyl CoA oxidase.

The evaluation of the activity of cardiac palmitoyl CoA oxidase shows that clofibrate (100 mg/Kg) was able to increase this parameter, suggesting that the dose of clofibrate employed was sufficient to activate PPAR signaling (Figure 1).

Cytosolic and nuclear expression of NF- κ B and I κ B.

The expression of p65 (NF- κ B) and I κ B α was evaluated by Western blot. Our results show that the cytoplasm content of NF- κ B was comparable between the experimental groups (Figure 2A). A different pattern of expression was observed in the nuclei, where MI-V rats underwent a rise in NF- κ B content. Conversely, clofibrate was able to prevent the increase induced by MI (Figure 2B).

Regarding I κ B α cytosolic content, our results show that MI-V animals exhibit a lower amount of this inhibitor subunit compared to control rats. Yet, clofibrate treatment in MI-C rats prevented the degradation of I κ B α , maintaining the values comparable to control (Figure 3A). The evaluation of I κ B α in the nuclei shows a lower expression in rodents undergoing the MI-V procedure compared to control rats. The level of I κ B α in the nuclei of MI-C animals is comparable to control rats (Figure 3B).

Effect of clofibrate on iNOS

We explored iNOS activity and expression, due to the capital relevance of its mediation of inflammatory and toxic processes (Heinzel et al. 2008). Our data shows that MI

promoted an increase in iNOS enzymatic activity, as well as a gain in protein and mRNA expression. PPAR α -stimulation by clofibrate prevented the latter changes and kept values closer to those of control rats (Figures 4 A, B, and C).

Evaluation of ICAM-1, MMP-2 and -9 and IL-6

The expression of ICAM-1, MMP-2 and -9 was evaluated by confocal microscopy and Western blot. As expected, left ventricles of MI-V rats exhibit a higher expression of the analyzed proteins; this change in expression, though, was partially prevented by clofibrate treatment (Figures 5, 6 and 7, * $p < 0.05$). The expression of IL-6 resembled that of other pro-inflammatory molecules (Figure 8). These results suggest that clofibrate exerts an anti-inflammatory effect during myocardial ischemia.

Infarct size

The evaluation of the area at risk (AAR) and area of necrosis (AON) shows that LAD produced an AAR comparable between MI-V and MI-C (Figure 9A). However, AON was reduced in those rats receiving clofibrate (MI-C), compared to rats given vehicle (Figure 9B).

***In silico* analysis**

Data analysis identified 3 motifs located in most of the genes studied: RAS, PPAR- α , iNOS, ICAM-1, VCAM, MMP-2 and -9 (Table 1). The first motif was located in 7 out of 13 genes explored. The second and third motifs show a higher incidence being present in 11 of the sequences studied. This coincidence may suggest a cross-regulation among PPAR- α and pro-inflammatory molecules.

Regarding the length of the motifs, the position respect to the beginning of transcription and the frequency of nucleotide appearance vary between the analyzed genes (Table 2).

Identified motifs were further analyzed using TESS database tools. The search revealed that Motif 1 is present in the glucocorticoid receptor (GR), lymphoid enhancer-binding factor 1 (LEF1), T cell factor 1 (TCF-1P), interferon regulatory factor-1 (IRF-1), and IRF2. Motif 2 was found to be a part of specificity protein 1 (SP1), LEF-1, TCF1P, and TGGCA-binding protein (NF-1). Also, motif 3 was located only in NF-1 (Table 3). It is worth mentioning that, the 3 motifs found belong to modulatory factors for lymphoid cellular lineage and they play an active role in the inflammatory process

Discussion

The data obtained in our study demonstrates that the stimulation of PPAR α by clofibrate (100 mg/Kg) promotes an anti-inflammatory cellular environment, which improves hemodynamics in myocardial infarction. It has been demonstrated that MI induces a marked inflammatory response (Hawkins et al. 1996). This response is elicited by an activation of NF- κ B, a rise in TNF- α , IL-1 β , IL-6, ICAM-1, and VCAM-1, and, as a

consequence, the migration of leukocytes to the myocardium. Once in the sub-endothelial space, the conversion of monocytes into macrophages also contributes to the progression of the inflammatory process and scar formation (Fuentes-Antras et al. 2014). Further supporting this, a correlation between hypertrophy, fibrosis, ventricular dysfunction, and cytokine release has been reported. Therefore, the reduction of the magnitude and duration of inflammation is desirable.

The role of PPAR α in the inflammatory response modulation at different stages has been reported for both cell culture and isolated tissues. This effect is achieved by regulating the expression of cytokines, cytokine receptors, and adhesion molecules (Duval et al. 2004). Delerive et al. reported that aortic vascular smooth muscle cells stimulated with WY14643 exhibited a higher expression of nuclear I κ B and higher inactivation of NF- κ B (Delerive et al. 2000). In our *in vivo* experimental model, clofibrate treatment was able to increase both cytoplasmic and nuclear expression of I κ B, and to concomitantly decrease the nuclear level of NF- κ B, thus supporting the evidence obtained by Delerive et al.

It has been reported that the cardiac production of proinflammatory cytokines, such as TNF- α or IL-6, is exclusively localized in ischemic tissue. Our data shows that clofibrate was able to protect the heart, evidenced as a smaller infarct size and it correlated with a lower production of IL-6. Therefore, it is reasonable to suggest that clofibrate exerts also an indirect anti-inflammatory effect preserving viability.

Here, we demonstrate that an *in vivo* PPAR α activation by clofibrate (100 mg/Kg) produces an increase in nuclear and cytoplasmic I κ B expression (Figures 3A and B), a

reduction in expression of not only NF- κ B (Figure 2B), but also of the molecular targets of NF- κ B activation: IL-6, MMP-2, MMP-9, and ICAM-1 (Figs. 5-8).

The mechanisms for PPAR α -induced lower inflammatory molecule production have not been fully elucidated. However, a relevant contributor in the process could be NO, since it is able to modulate the expression of ICAM-1 and VCAM-1 (Carreau et al. 2011). Previous and current evidence obtained by our group reveal that clofibrate increases the expression and activity of eNOS and NO bioavailability (Ibarra-Lara et al. 2010; Ibarra-Lara et al. 2012), suggesting that replenishing NO levels in AMI would decrease the expression of adhesion molecules.

Our data also illustrates that PPAR α stimulation decreases the participation of iNOS. It has been reported that a gain in the activity and/or expression of iNOS is related to cytotoxic effects. Therefore, the employment of strategies to decrease iNOS participation is promising. As shown in figure 4, clofibrate treatment decreased proteic and mRNA expression of iNOS; we point to NF- κ B as the mediator which leads to the clofibrate-induced decreased participation of iNOS. As previously reported, NF- κ B binds to iNOS promoter sequence resulting in an increased production of its mRNA (López-Bojórquez et al. 2004). Since clofibrate decreased NF- κ B nuclear translocation, it is reasonable to suggest that clofibrate induced a lower iNOS expression through NF- κ B.

Concerning the analysis *in silico*, we aimed at exploring if bioinformatics methods would show the level of interaction between PPAR α and target genes. The data shows that motifs are close to the beginning of transcription and are long enough to work as promoters, as reported by Spontaneo and Cercone. The latter characteristic identifies

transcription factors in nucleotide islands (Spontaneo and Cercone 2011). Plutzky reported that PPAR response elements (PPRE) are present in inflammation-related genes, suggesting that those motifs could favor transcription and its regulation (Plutzky 2011).

Our study shows that, compared to other nucleotides, thymine has the highest incidence, while cytosine exhibits the lowest. These parameters could be related to regulation of transcription and DNA methylation, which is performed by DNA methyl transferases (DNMTs) on CpG islands (Webster et al. 2013).

The data analysis shows that motifs shared by studied genes belong to transcription factors. Motif 1 was found in GR, LEF-1, TCF-1(P), IRF1, and IRF2. Interestingly, GR belongs to the nuclear receptor super-family; it works as a transcription factor, and binds to other transcription factors inhibiting NF- κ B exerting an anti-inflammatory effect (Kassel and Herrlich 2007). Factors like LEF-1 and TCF-1(P) have been related to the regulation and function of lymphoid lineage. Actually, TCF-1 represses the expression of IL-17, a pro-inflammatory cytokine (Ma et al. 2011). Motif 1 was also found in IRF1 and IRF2, interferon regulating factors that function as transcription factors. Blanco et al. reported that both (IRF-1 and -2) regulate the expression of cyclooxygenase-2 (COX-2), an inducible, pro-inflammatory enzyme (Blanco et al. 2000).

As for Motif 2, we found that it was present in LEF-1, TCF-1(P), Sp1 and NF-1. Interestingly, Sp1 belongs to the Sp/KLF family of transcription factors and participates in bone inflammatory processes (Duttenhoefer et al. 2014). Taken together, the *in silico*

evidence suggests that PPAR α exerts a regulatory role in the inflammatory pathways. Further experimental studies are still needed to confirm this.

Conclusion

The stimulation of PPAR α prevents the expression of pro-inflammatory molecules, preserving cardiac functionality and viability posterior to AML.

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Draft

Legends

Figure 1. Effect of clofibrate on palmitoyl CoA oxidase. The activity was measured in myocardial infarcted area from control, myocardial infarcted (MI) + vehicle (MI-V), and MI + clofibrate (MI-C) rats. The bars represent the mean $\pm$ standard error of the mean of 6 different experiments. * $p < 0.05$ vs. control ANOVA-Tukey, # $p < 0.05$ vs. MI-V ANOVA-Tukey.

Figure 2. Effect of clofibrate on nuclear factor- κ B expression (NF- κ B). The expression of NF- κ B in (A) cytosol and (B) nuclei from myocardial infarcted area of control, myocardial infarcted (MI) + vehicle (MI-V), and MI + clofibrate (MI-C) rats was assessed by Western blot. The bars represent the mean $\pm$ standard error of the mean of 6 different experiments. * $p < 0.05$ vs. control ANOVA-Tukey, # $p < 0.05$ vs. MI-V ANOVA-Tukey.

Figure 3. Effect of clofibrate on inhibitor- κ B expression (I κ B). The expression of I κ B in (A) cytosol and (B) nuclei from myocardial infarcted area of control, myocardial infarcted (MI) + vehicle (MI-V), and MI + clofibrate (MI-C) rats was assessed by Western blot. The bars represent the mean $\pm$ standard error of the mean of 6 different experiments. * $p < 0.05$ vs. control, ANOVA-Tukey, # $p < 0.05$ vs. MI-V, ANOVA-Tukey.

Figure 4. Effect of clofibrate on inducible nitric oxide synthase (iNOS). (A) The enzymatic activity, (B) proteic expression, and (C) mRNA expression of iNOS was evaluated in myocardial infarcted area of control, myocardial infarcted (MI) + vehicle (MI-V), and MI + clofibrate (MI-C) rats. The bars represent the mean $\pm$ standard error of the mean of 6 different experiments. * $p < 0.05$ vs. control, ANOVA-Tukey, # $p < 0.05$ vs. MI-V ANOVA-Tukey.

Figure 5. Effect of clofibrate on the expression of matrix metalloproteinase-2 (MMP-2) in ischemic myocardium. The expression of MMP-2 was evaluated by (A) confocal microscopy (60X) and (B) Western blot in myocardial infarcted area of control, myocardial infarcted (MI) + vehicle (MI-V), and MI + clofibrate (MI-C) rats. The bars represent the mean $\pm$ standard error of the mean of 6 different experiments. * $p < 0.05$ vs. control, ANOVA-Tukey.

Figure 6. Effect of clofibrate on the expression of matrix metalloproteinase-9 (MMP-9) in ischemic myocardium. The expression of MMP-9 was evaluated by (A) confocal microscopy (60X) and (B) Western blot in myocardial infarcted area of control, myocardial infarcted (MI) + vehicle (MI-V), and MI + clofibrate (MI-C) rats. The bars represent the mean $\pm$ standard error of the mean of 6 different experiments. * $p < 0.05$ vs. control, ANOVA-Tukey, # $p < 0.05$ vs. MI-V ANOVA-Tukey.

Figure 7. Effect of clofibrate on the expression of intercellular adhesion molecule-1 (ICAM-1) in myocardial ischemic rats. The expression of ICAM-1 was evaluated by (A) confocal microscopy (100X) and (B) Western blot in myocardial infarcted area of control, myocardial infarcted (MI) + vehicle (MI-V), and MI + clofibrate (MI-C) rats. The bars represent the mean $\pm$ standard error of the mean of 6 different experiments. * $p < 0.05$ vs. control, ANOVA-Tukey, # $p < 0.05$ vs. MI-V ANOVA-Tukey.

Figure 8. Effect of clofibrate on the expression of interleukin-6 (IL-6) in myocardial ischemic rats. The expression of IL-6 was evaluated by ELISA in serum from control, myocardial infarcted (MI) + vehicle (MI-V), and MI + clofibrate (MI-C) rats. The bars represent the mean $\pm$ standard error of the mean of 6 different experiments. * $p < 0.05$ vs. control, ANOVA-Tukey, # $p < 0.05$ vs. MI-V ANOVA-Tukey.

Figure 9. Evaluation of the infarct size. The effect of clofibrate on the extension of the area at risk (AAR) and area of necrosis (AON) was evaluated in the left ventricle of control, myocardial infarcted (MI) + vehicle (MI-V), and MI + clofibrate (MI-C) rats. The bars represent the mean $\pm$ standard error of the mean of 6 different experiments. * $p < 0.05$ vs. control, ANOVA-Tukey, # $p < 0.05$ vs. MI-V ANOVA-Tukey.

C)

iNOS 130 KDa
 β -Actin 43 KDa

Table 1. List of the 5’ untranslated region (5’UTR) of genes analyzed using MEME program. The table shows the presence of identified motifs into 5’UTR genes analyzed.

Gene	Motif 1	Motif 2	Motif 3
ICAM-1		*	*
VCAM-1	*	*	
MMP-2		*	*
MMP-9		*	*
PPAR α		*	*
iNOS	*	*	*
Renin		*	*
Angiotensinogen	*		*
ACE		*	
ACE-2	*	*	*
Angiotensin II AT1 receptor	*	*	*
Angiotensin II AT2 receptor	*		*
Mas receptor	*	*	*

Table 2. Motifs sequences in 5' untranslated regions (5'UTR) obtained by MEME.

Motif	Sequence	Length	Position
1	TTCTTCTTCTTTATTTTTCTTCTTAAGTGAAGCATTT	40	-266
2	AGTGCAAAGCCAGGG	15	-358
3	CTGGAAGACTG	11	-262

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Table 3. Transcription factors of TESS database.

Motif 1	Motif 2	Motif 3
GR	Sp1	NF-1
LEF-1	LEF-1	
TCF-1P	TCF-1P	
IRF1	NF-1	
IRF2		

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