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Gene Expression Related to Oxidative Stress in the Heart of Mice after Intestinal Ischemia

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Abstract

Background: Intestinal ischemia-reperfusion is a frequent clinical event associated to injury in distant organs, especially the heart.

Objective: To investigate the gene expression of oxidative stress and antioxidant defense in the heart of inbred mice subjected to intestinal ischemia and reperfusion (IR).

Methods: Twelve mice (C57BL / 6) were assigned to: IR Group (GIR) with 60 minutes of superior mesenteric artery occlusion followed by 60 minutes of reperfusion; Control Group (CG) which underwent anesthesia and laparotomy without IR procedure and was observed for 120 minutes. Intestine and heart samples were processed using the RT-qPCR / Reverse transcriptase-quantitative Polymerase Chain Reaction method for the gene expression of 84 genes related to oxidative stress and oxidative defense (Student's "t" test, $p < 0.05$).

Results: The intestinal tissue (GIR) was noted to have an up-regulation of 65 genes (74.71%) in comparison to normal tissue (CG), and 37 genes (44.04%) were hyper-expressed (greater than three times the threshold allowed by the algorithm). Regarding the remote effects of intestinal I/R in cardiac tissue an up-regulation of 28 genes (33.33%) was seen, but only eight genes (9.52%) were hyper-expressed three times above threshold. Four (7.14%) of these eight genes were expressed in both intestinal and cardiac tissues. Cardiomyocytes with smaller and pyknotic nuclei, rich in heterochromatin with rare nucleoli, indicating cardiac distress, were observed in the GIR.

Conclusion: Intestinal I/R caused a statistically significant over expression of 8 genes associated with oxidative stress in remote myocardial tissue. (Arq Bras Cardiol. 2014; 102(2):165-174)

Keywords: Gene Expression; Mice; Oxidative Stress; Intestines / pathology.

Introduction

Tissue ischemia and reperfusion (IR), and the cell and humoral changes that follow have a great impact in human homeostasis[1]. Although reactive oxygen species (ROS) are involved in homeostasis, if a number greater than the natural limits of cell defense of antioxidants and sweepers is present, an unbalance will be seen, known as oxidative stress[2]. Although the oxygen is a critical substrate for relieving ischemia, it paradoxically acts as a harmful metabolite during the reperfusion period[2-5], creating a predisposition for injury in distant organs, in addition to local lesions[6].

IR, and the subsequent oxidative stress, is associated to clinical and surgical conditions, such as: sepsis, artery occlusion, cardiac arrest, thrombosis, embolism, vasospasm, tumor, organ transplantation, organ excision, cerebrovascular accident and myocardial infarction[2,6-8].

Oxidative stress in the cardiovascular system predisposes to severe cardiomyocyte and cardiac vessel injury, changing their contraction and relaxation mechanisms, having as consequence their disadaptation and impaired function[6].

Gene expression assessment associated with oxidative stress in distant organs is a new tool for clarifying different aspects of the phenomenon, both related to diagnosis and monitoring, and therapeutic procedure results[9,10]. A recent technological resource makes it possible to assess a large number of genes in a safe and relatively simple way, using RT-qPCR (Reverse transcriptase–quantitative Polymerase Chain Reaction) method[11-14]. Thus, the genomic response of the heart to the oxidative stress caused by renal, hepatic, and mesenteric ischemia, infrarenal aortic occlusion or lower limb ischemia has been described in animal models[13-14].

Considering splanchnic ischemia, especially of the small intestine, is a severe event, and that in addition to its local manifestations it has a systemic repercussion, this paper proposes to assess the expression of 84 genes related to

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the oxidative stress and the antioxidant defense in the cardiac tissue after intestinal ischemia and reperfusion in an inbred mice model.

Methods

The research was approved by the Research Ethics Committee of Hospital São Paulo, bonded to Universidade Federal de São Paulo-UNIFESP under number 1379-1308. The project was ratified by the Research Ethics Committee of Universidade Federal da Grande Dourados-UFGD (protocol 306/08). Experimental procedures followed international norms about animal research and the guidelines of the Brazilian Society of Lab Animal Science (SBCAL). The animals were kept according to the Guide for Caring and Using Lab Animals (Institute of Lab Animal Research, 1996).

Sample

The animals were kept under controlled conditions of temperature and noise, and had a 12-hour cycle of light and darkness. They had free access to water and food adequate to the species. Surgical procedures were performed in the Experimental Surgery labs of the Surgical Department of Universidade Federal da Grande Dourados (UFGD). Twelve adult male mice were selected (C57BL/6 strain), with an average weight of 35 grams. They were supplied by the Center for the Development of Experimental Models for Medicine and Biology UNIFESP (CEDEME) and randomly assigned to either one of these two groups: Ischemia and Reperfusion Group (GIR), where six animals were subjected to superior mesenteric artery occlusion for 60 minutes followed by 60 minutes of reperfusion; and Control Group (GC), where six animals were subjected to median laparotomy under anesthesia, with no vascular clamping of the intestine, and observed for 120 minutes.

Anesthetic and surgical procedures

The mice were administered the following anesthetics intramuscularly: ketamine hydrochloride (44 mg.Kg^{-1}, Ketamina Agener - União Química, São Paulo), xylazine hydrochloride (2.5 mg.Kg^{-1}, Calmium - União Química, São Paulo) and acepromazine (0.75 mg.Kg^{-1}, Acepran - Rhobifarma). Room temperature was kept at 38°C. The surgical procedure included median laparotomy, and after opening of the peritoneal cavity, abdominal viscera were kept away and identified, and the superior mesenteric artery and vein were isolated and occluded by a microsurgery clamp (metal vascular clip). Artery occlusion was confirmed by pale coloration of the intestine and no pulsing of the artery. After 60 minutes of vascular occlusion, the metal clamp was removed, and reperfusion was initiated, characterized by the return of artery pulsing and different coloration.

Material collection procedure

After the reperfusion period in the GIR group or the observation period in the GC group was finished, samples of the small intestine of the mice were collected for the assessment of ischemia effects and gene expression. Sample collection

was performed under anesthesia. Subsequently, the heart was removed, still beating, and sectioned lengthwise at the larger axis, by the ventricles. Heart fragments were imbided into 10% formaldehyde individually and subjected to standard optical microscope histological processing for hematoxiline and eosin (HE). Another lengthwise fragment of the heart and a 30 mm segment of the intestine were carefully washed with saline solution, wrapped in aluminium foil, labeled and placed in cryogenic vials containing liquid nitrogen (-196°C). These samples were taken to the Molecular Biology Lab of the Gynecology Department - UNIFESP for gene expression processing.

Gene expression procedures

The RNA of the 84 genes related to the effect of the oxidative stress and antioxidant defense (Table 1) was extracted from the small intestine and heart samples of mice in the GC and GIR using TRIzol® (Life Technologies, Grand Island, NY, USA) reagent, and purified by inverse measures (Rneasy MiniKit Qiagen, Co - USA). The concentration of total RNA samples was determined by spectrophotometry, and result quality was assured by the same 2% agarose gel analysis. The first complementary DNA chain (cDNA) was synthesized using 1 μg of total RNA and RT^2 First Strand kit (SABiosciences). Identical amounts of cDNA and Master Mix SYBR® Green qPCR Mastermix (SABiosciences, QIAGEN Company) were distributed to each PCR array well containing portions of specific genes, which had been previously selected. PCR was performed according to manufacturer's instructions in 96 wells for the expression of 84 genes related to oxidative stress, five endogenous control genes used for measuring PCR array information (ACTB, Gapdh, Hsp90ab1, Hprt1, Gusb), and one negative control for checking for potential contamination with genomic DNA. The negative control primary sample detected non-transcribed and repetitive genomic DNA with a high level of sensitivity. Three reverse transcription control (RTC) wells were used to check for RT reaction efficiency with qPCR test, which aims at detecting patterns synthesized by the control RNA of the first strand synthesis kit. Replication of positive PCR controls (PPCs) was used to determine the efficiency of the polimerase chain reaction. These controls use an artificial DNA sequence predefined in the detection process. Replicated control wells (RTC and PPC) also assess the consistency among wells and plates. The equipment software (MxPro Equipment Real Time Systems, Stratagene, GE, Co) calculated the values of the beginning of the cycle (Ct) for all genes under analysis. At last, the software compared the pair by calculating gene expression change from the pure initial cycle, using [$2^{-\Delta\Delta C}$] method. The method used for determining the relative expression of interest genes in this study was shown in the data analysis table of PCR Array v3.3 (PCR Array Data Analysis v3.3-SABiosciences – Qiagen, Company)[15].

Statistical Analysis

Each sample was assessed in triplicate for gene expression data. Student's t-test (p < 0.05) was used to validate the homogeneity of each gene expression reaction. For comparison between the two groups, the computer

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#	Families	Number of genes	Genes investigated
1	Glutathione Peroxidases (GPX):	10	Gpx1, Gpx2, Gpx3, Gpx4, Gpx5, Gpx6, Gpx7, Gpx8, Gstk1, Gsr.
2	Peroxiredoxins (TPX):	8	Ehd2, Prdx1, Prdx2, Prdx3, Prdx4, Prdx5, Prdx6, Prdx1rs1.
3	Peroxidases	16	Aass, Apc, Cat, Ctsb, Duox1, Epx, Lpo, Mpo, Ptgs1, Ptgs2, Rag2, RGD1560658 (Serpinb1b), RGD1565187 (Kif9), Slc41a3, Tmod1, Tpo.
4	Reactive Oxygen Species	16	Ccs, Cyba, Fmo2, Il19, Il22, Ncf2, Nos2, Nox1, Nox4, Noxa1, Noxo1, Recql4, Scd1, Sod1, Sod2, Sod3.
5	Oxidative Stress	22	Als2, Apoe, Ercc2, Ercc6 Gab1, Idh1, Mpp4, Nqo1, Nudt15, Nxn, Park7, Ppp1r15b, Prnp, Psmb5, Srxn1, Txnip, Txnrd1, Txnrd2, Txnrd3, Ucp3, Xpa, Zmynd17.
6	Oxygen Carriers	12	Aqr, LOC367198 (Atr), Cygb, Dnm2, Fancc, Hbq1, Slb (Ift172), Mb, Ngb, Slc38a1, Vim , Xirp1.

program calculated the quantification cycle variation (Ct) of the study group in relation to the quantification cycle (Ct) of the control group expressed in the logarithm basis (2) by the $2^{(-Delta\ Delta\ Ct)}$ formula. The gene expression results are shown as positive expression (GIR higher than GC) or negative expression (GIR lower than GC). The numbers represent how many times each gene was expressed, with the positive sign (+, higher) or the negative sign (-, lower). The software calculated results three times higher (hyper-expression) or three times lower (hypo-expression) than the threshold allowed by the algorithm for statistical significance ($p < 0.05$).

Results

Gene Expression Assessment

From the 84 genes assessed in the intestine, 65 genes (74.71%) had an up-regulation, and out of these, 37 genes (44.04%) were hyper-expressed, that is, the expression was three times higher than the threshold established by the algorithm in comparison to the control group (Chart 1).

The cardiac tissue showed an up-regulation of 28 genes (33.33%) after intestinal I/R (Table 2). Only 8 genes (9.52%) were three times above the algorithm threshold (Table 3 and chart 2). Four genes (4.76%) were positively and simultaneously expressed in both intestinal and cardiac tissues (Table 4).

Histological Assessment

In the qualitative histological analysis, heart tissue samples of the animals from both groups were formed by strips of cardiac muscle fibers separated from one another by type I collagen fibers. These strips contained elongated cardiac muscle cells with one or two nuclei at the center, and transversal grooves in the cytoplasm. Around the cardiomyocytes, there are connective tissue cells, type III collagen fibers and blood capillary.

The GC was noted to have cardiomyocytes with one or two large nuclei and very marked nucleoli (Figure 1). The GIR (one hour of ischemia and one hour of reperfusion) was noted to have smaller cardiomyocytes with pyknotic and darker nuclei, rich in heterochromatin with rare nucleoli, indicating cardiac distress (Figure 2).

Discussion

Data obtained from the research on oxidative stress and cell antioxidant response have showed that, in a short term model of intestinal ischemia (one hour of ischemia followed by one hour of reperfusion) in inbred mice, certain genes were positively expressed in the cardiac tissue (Table 2 and chart 1).

A global analysis confirms that intestinal ischemia followed by reperfusion is a phenomenon closely related to the generation and modulation of reactive oxygen species. Local changes in the intestine promoted humoral and cell responses which, after being disseminated through blood flow, affected the genomic apparatus of the cardiac tissue, inducing gene expression responses related to the generation and modulation of ROS.

The aminoadipate-semialdehyde synthase (Aass) gene encodes a catalyzing peroxidase protein whose substrates are hydrogen peroxide and organic hydroperoxide. It can be found in the majority of the tissues, functioning as a bifunctional enzyme which degradates lysine up to acetyl coenzyme A (acetyl-CoA) through two different metabolic pathways (saccharopine and pipecolic acid)[16,17]. Lysine causes lipid peroxidation, thereby reducing the concentration of glutathione peroxidases and harming cell antioxidant defenses. It acts by inhibiting Na^+/K^+-ATPase with changes in cell membrane ionic exchange mechanisms, inducing oxidative stress[18,19]. Lysine degradation by acetylation influences myocardial motility, increasing the actin sliding speed on myosin in comparison to their respective non-acetylated isoforms[19]. In the light of the known activities of the enzyme encoded by the Aass gene, it is possible to infer that its hyper-expression in the cardiac tissue is associated with protection of cardiac tissue against the harmful action of ROS.

Prostaglandin-endoperoxide synthase (Ptgs-2) is a peroxidase that converts the arachidonic acid intro prostaglandin and it is associated with injuries caused by the inflammatory process and cell proliferation[20]. It has two functions in the inflammatory process: initially, it contributes to the onset of the inflammatory response, and then it acts in process resolution[21,22]. Ptgs2 is not detected in normal cells, but it is present in responses
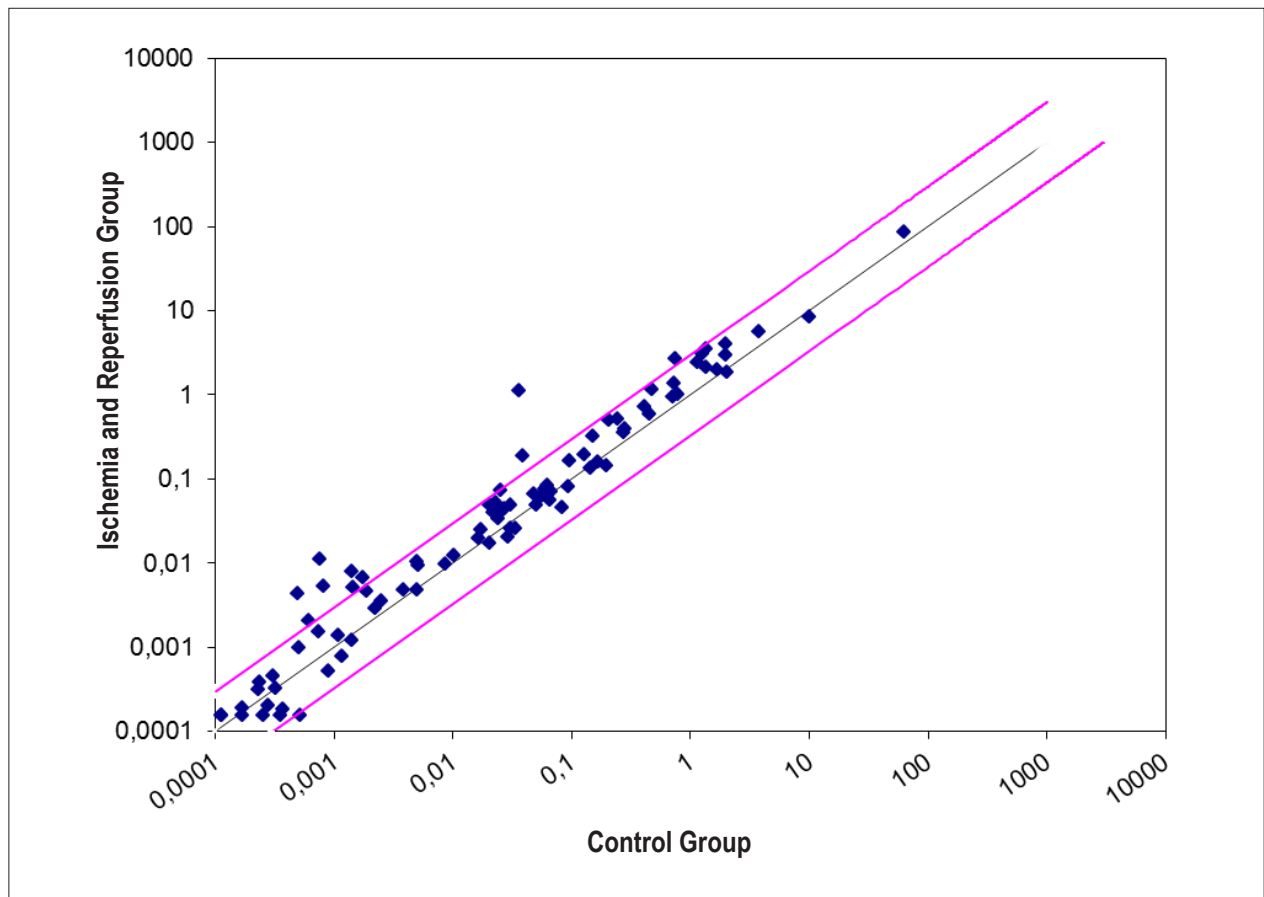
Chart 1 - *Disposition of the expression of 84 genes in the cardiac tissue related to intestine ischemia and reperfusion in the animals of GC and GIR groups. The black line indicates normal expression; the pink lines indicate three times higher than the gene expression allowed by the algorithm.*

to inflammatory cytokines in different types of cells and oxidative stress situations. Its presence has been reported in the myocardium in many different diseases which resulted in cardiac failure. In the context of this research paper, the hyper-expression of Ptgs2 gene has showed that myocardial tissue is sensitive to intestinal ischemia, reacting in a defensive way against oxidative stress[22].

Xenobiotic neutralization processes, including amines, sulfides and some phosphorus and selenium compounds, are performed by a mono-oxygenase enzyme (Fmo2) containing flavin nucleotide. The reaction depends on the presence of NADPH (Nicotinamide adenine dinucleotide phosphate) hydrogenase. In the absence of NADP (nicotinamide adenine dinucleotide phosphate) Fmo2, it would work as NADPH oxidase consuming NADPH and causing oxidative stress upon the excessive production of hydrogen peroxide[23]. Mammals express five different mono-oxygenases, and Fmo2 isoform is the most prevalent. A study in humans has associated Fmo2 with a higher resistance to the oxidative stress caused by environmental factors[24,25]. In this research paper, Fmo2 gene hyper-expression suggests that intestinal ischemia induces protein encoding for antioxidant defense of the cardiac tissue.

NADPH oxidase (Nox) is a group of seven oxidase enzymes with intracellular and perinuclear manifestation, present in different tissues. Its presence is quite relevant in the cardiovascular tissue, although with no clear distinction between myocytes and other vascular components. Nox4 is the isoforms involved in ROS production, especially hydrogen peroxide and superoxide anion in a smaller scale[26]. It is involved in oxygen detection, vasomotor control, cell proliferation, differentiation, migration, apoptosis, senescence, fibrosis and angiogenesis[27]. Inflammatory cytokines, including TNF-alpha, function as Nox4 gene expression modulators[28]. Both play an important role in the pathogenesis of cardiovascular diseases, stimulating the extrinsic pathway in the formation of a death inducing signaling complex, resulting in the activation of the caspase cascade. The correlation of these in cardiovascular diseases is clear (atherosclerosis, blood hypertension, cardiac failure, and ischemic cerebrovascular accident)[26,28,29]. Its hyper-expression confirms intestinal ischemia/reperfusion had a harmful effect on the heart. This effect can also be morphologically confirmed by the changes identified in HE stained slides (Figure 2).

Apolipoprotein E (Apoe) is fundamental for the regular metabolism of triglycerides and cholesterol lipoprotein

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Table 2 - Distribution of 28 genes out of the 84 genes investigated in the heart with positive expression (+) or negative expression (-) 2^(- Delta Delta Ct) in the animals subjected to a 60-minute ischemia in the small intestine followed by a 60-minute reperfusion in comparison to the control group that was not subjected to ischemia/reperfusion; (* = significant p-value < 0.05)

#	Gene Basis	Gene Symbol	Gene Name	GIR Intestine	P-value	GIR Heart	P-value
1	NM_013930	Aass	Aminoadipate-semialdehyde synthase	+19.63*	0.000001	+6.65*	0.01209
2	NM_009696	Apoe	Apolipoprotein E	+1.76*	0.002533	+3.60*	0.01737
3	NM_007985	Fancc	Fanconi anemia. complementation group C	+7.42*	0.000015	+1.87*	0.04313
4	NM_018881	Fmo2	Flavin containing monooxygenase 2	+1.52*	0.019633	+4.93*	0.00772
5	NM_010343	Gpx5	Glutathione peroxidase 5	+5.10*	0.001774	+0.50*	0.03717
6	NM_027127	Gpx8	Glutathione peroxidase 8 (putative)	+1.43	0.072334	+2.19*	0.01271
7	NM_010344	Gsr	Glutathione reductase	+1.64	0.149896	+2.34*	0.02997
8	NM_080420	Lpo	Lactoperoxidase	+23.88*	0.00002	-2.28*	0.00326
9	NM_010877	Ncf2	Neutrophil cytosolic factor 2	+4.31*	0.000018	+2.43*	0.02876
10	NM_010927	Nos2	Nitric oxide synthase 2. inducible	+4.71*	0.000117	+1.31*	0.00129
11	NM_015760	Nox4	NADPH oxidase 4	+12.47*	0.000005	+5.73*	0.02561
12	NM_172204	Noxa1	NADPH oxidase activator 1	+3.06*	0.000083	+1.49*	0.00072
13	NM_027988	Noxo1	NADPH oxidase organizer 1	+1.33*	0.000177	+2.10*	0.03623
14	NM_008750	Nxn	Nucleoredoxin	+1.98*	0.002268	+2.53*	0.036
15	NM_133819	Ppp1r15b	Protein phosphatase 1. regulatory (inhibitor) subunit 15b	+1.06	0.962143	+1.99*	0.04908
16	NM_012021	Prdx5	Peroxiredoxin 5	-1.54*	0.008534	+2.14*	0.01926
17	NM_011186	Psmb5	Proteasome (prosome. macropain) subunit. beta type 5	+1.21	0.078442	+1.32*	0.00488
18	NM_008969	Ptgs1	Prostaglandin-endoperoxide synthase 1	+1.56*	0.012124	+2.94*	0.00004
19	NM_011198	Ptgs2	Prostaglandin-endoperoxide synthase 2	+10.07*	0.000003	+3.94*	0.00691
20	NM_009020	Rag2	Recombination activating gene 2	+15.79*	0.000293	+3.42*	0.04444
21	NM_009127	Scd1	Stearoyl-Coenzyme A desaturase 1	+6.85*	0.00042	+31.73*	0.000099
22	NM_134086	Slc38a1	Solute carrier family 38. member 1	+4.59*	0.000106	+8.86*	0.01516
23	NM_013671	Sod2	Superoxide dismutase 2. mitochondrial	+1.47*	0.009249	-1.80*	0.02156
24	NM_011435	Sod3	Superoxide dismutase 3. extracellular	+2.51*	0.002347	+1.28*	0.00081
25	NM_021883	Tmod1	Tropomodulin 1	+15.01*	0.000161	-1.05*	0.00619
26	NM_023719	Txnip	Thioredoxin interacting protein	+1.87*	0.00026	+2.02*	0.00127
27	NM_009464	Ucp3	Uncoupling protein 3 (mitochondrial. proton carrier)	+15.85*	0.000102	+1.62*	0.01441
28	NM_011701	Vim	Vimentin	+2.20*	0.000155	+2.59*	0.01509

Table 3 - Distribution of 8 hyper-expressed genes 2^(- Delta Delta Ct) out of the 84 genes investigated in the heart compared to the genes expressed in the intestine of the animals subjected to a 60-minute ischemia in the small intestine followed by a 60-minute reperfusion in comparison to the control group that was not subjected to ischemia/reperfusion; (* = p-value < 0.05)

#	Gene Basis	Gene Symbol	Gene Name	GIR Intestine	P-value	GIR Heart	P-value
1	NM_013930	Aass	Aminoadipate-semialdehyde synthase	+19.64	0.000001*	+6.65	0.012092*
2	NM_009696	Apoe	Apolipoprotein E	+1.94	0.002533*	+3.6	0.017365*
3	NM_018881	Fmo2	Flavin containing monooxygenase 2	+1.6	0.019633*	+4.93	0.007719*
4	NM_015760	Nox4	NADPH oxidase 4	+3.13	0.000005*	+5.73	0.025614*
5	NM_011198	Ptgs2	Prostaglandin-endoperoxide synthase 2	+14.6	0.000003*	+3.94	0.006908*
6	NM_009020	Rag2	Recombination activating gene 2	+10.76	0.000293*	+3.42	0.044442*
7	NM_009127	Scd1	Stearoyl-Coenzyme A desaturase 1	+1.62	0.000420*	+31.73	0.000099*
8	NM_134086	Slc38a1	Solute carrier family 38. member 1	-1.29	0.000106*	+8.86	0.015159*

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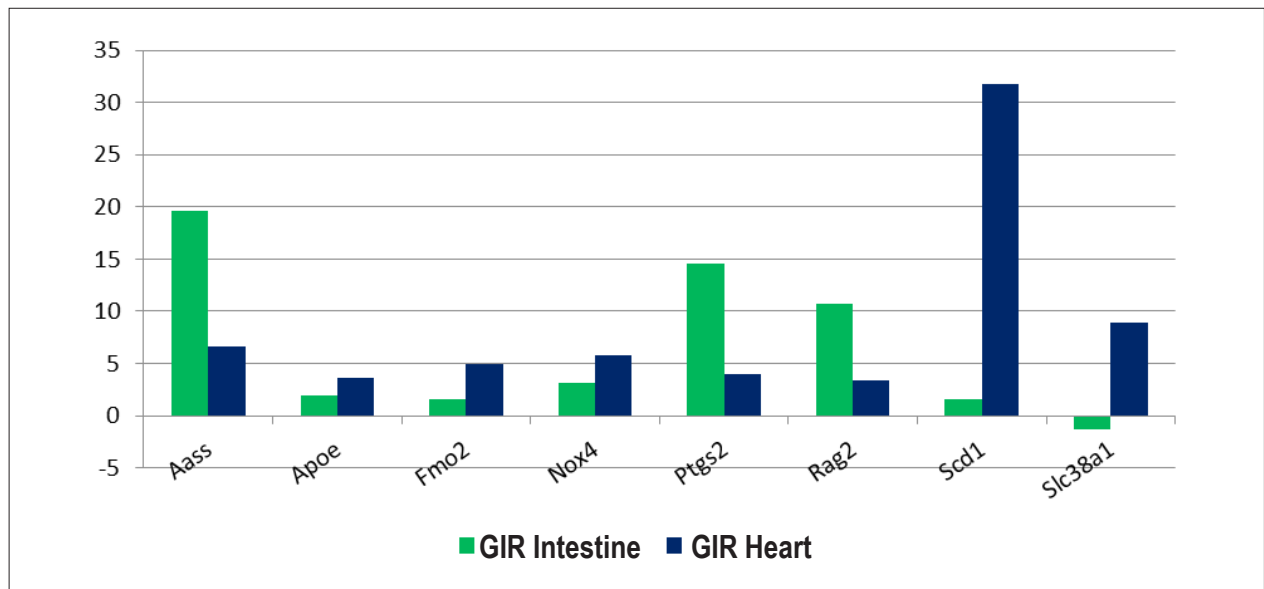
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Chart 2 - *Eight genes related to oxidative stress and expressed three times above the threshold allowed by the algorithm in intestine and cardiac tissues.*

Table 4 - **Amount and percentage of genes hyper-expressed with statistically significant values and simultaneous occurrence in the intestine and heart tissues**

	GIR Intestine	GIR Heart	Simultaneous occurrence
Genes considered to be significant (p < 0.05)	65 (74.71%)	28 (33.33%)	24 (28.57%)
Genes considered to be hyper-expressed	37 (44.04%)	8 (9.52%)	4 (4.76%)

components. Primarily produced by the liver and macrophages, it's a cholesterol carrier, protects vessel endothelial cells, has an apparent function in neurological diseases, and has *in vitro* immunomodulator functions, decreasing lymphocyte proliferation after mitogenic stimulus[30]. This alipoprotein has been reported to act against ischemia, by acting as an antioxidant, protecting cells from different tissues, such as the endothelium against the toxic effect of hydrogen peroxide[31-34]. Considering the activities of Apoe in different tissues, it is possible to construct a hypothesis on the fact there is a protecting response against the harmful effects of ROS on the heart after oxidative stress on our experimental model.

Rag2 gene (recombination-activating gene 2) encodes one of the peroxidases that play an important role in rearranging and recombining immunoglobulin and T cell molecular receptor genes[27]. It is a complex multiprotein, which mediates the DNA cleavage phase during recombination. RAG1 and RAG2 proteins are crucial for the maturation of bursal (B) and timic (T) lymphocytes. Both cell types are fundamental for immune system adaptation[35]. Peroxidases are known to influence oxidative stress modulation[35,36]. Rag2 hyper-expression can be associated with the need of adjustments in cardiac cell replication due to the presence of excessive ROS, especially hydrogen peroxide and organic hydroperoxides, showing a protecting action against the harmful effects of ROS.

The protein encoded by Scd1 gene (Stearoyl-Coenzyme A desaturase 1) catalyzes unsaturated fatty acid synthesis and its major byproduct is oleic acid, formed by stearic acid desaturation, and it is part of the enzyme family related to reactive oxygen species[37]. Scd1 deficiency increases fatty acid oxidation rate in the liver and skeletal muscle. The higher this rate is, the higher the chances that Scd1 deficiency changes cardiac metabolism, influencing in the proportion of energetic substrate among fatty acids and glucose available to the heart[38]. Incompatibility between the absorption and the use of long chain fatty acids by the myocardium results in abnormally increased concentration of intracellular fatty acid, inducing myocardium dysfunction[37]. In this research paper, hyper-expression of the gene in the cardiac tissue may be related to the need of a greater use of fats in the lieu of the glucose dependent oxidizable substrate, in an attempt to repair the cardiac metabolic unbalance.

The protein encoded by Slc38a1 gene (Solute carrier family 38) is an amino acid carrier predominantly present in the heart, brain and placenta. Solute carriers are proteins from the eukaryotic membrane that control the absorption and the outflow of different solutes, including amino acids, sugars and drugs[39]. Amino acids are necessary for different important cell biological processes, such as in the production of thiol glutathione (GSH) catalyzed by the presence of cysteine, which is also necessary to other reactions in the myocardial

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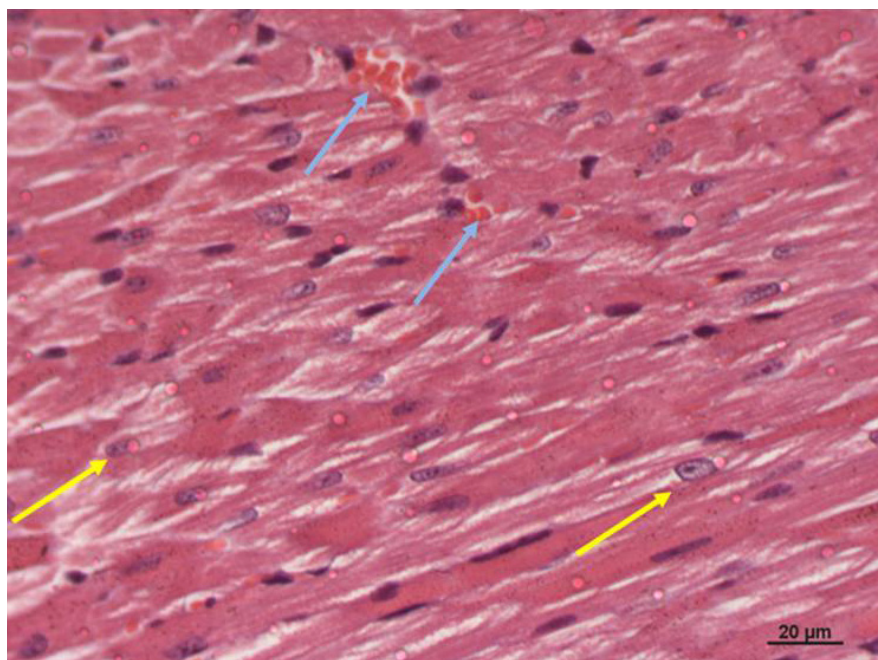
Figure 1 - *Photomicrography of the left ventricle of a mouse in the Control Group (GC). Please note large volume and euchromatic nuclei of cardiomyocytes centralized in the cell (yellow arrows). Preserved vessels with no red blood cell extravasation (blue arrows). Cardiac fibers correctly positioned. (HE 400x).*
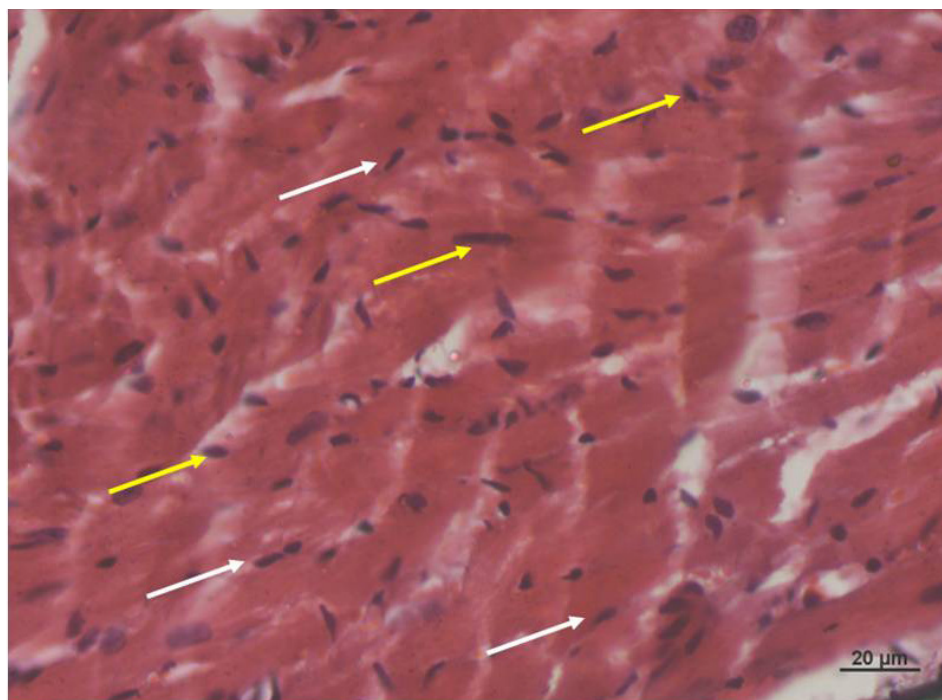
Figure 2 - *Photomicrography of the left ventricle of a mouse in the Ischemia and Reperfusion Group (GIR). Please note smaller volume and pyknotic nuclei (yellow cell) of cardiomyocytes in the edge of the cell (white cell). Swollen cells and cardiac fibers in a disorganized direction. (HE 400x).*

tissue and is provided by the Slc38a1 carrier protein[40]. The duration of the oxidative stress stimulates its uptaking, causing increased Slc38a1 expression in the myocardium of mammals, consequently with a higher expression of GSH[39,40]. In this study, hyper-expression of Slc38a1 corroborates with the indication of similar action in other tissues and shows that its presence can implicate in a protecting action in the energy transportation mechanism of the myocardium cell.

The rationale of this research paper was developing the gene response profile of the cardiac tissue when subjected to stimuli from distant organs affected by oxidative stress. In the light of the unprecedented nature of the results and the complexity of the gene expression, the results can only be confirmed with a subsequent proteomic analysis, where protein identification and quantification in the tissue may provide resources to determine whether the gene expression acted as a real stimulus for protein encoding or whether the gene expression is related to signaling or modulation of other oxidative stress response metabolic pathways. As a starting point to future research, the mapping activity performed allowed eight genes to be identified, which showed to be more responsive to oxidative stress and can, therefore, be initially targeted as new study subject. This research line is very promising, because gene expression understanding may allow evolution monitoring procedures and treatment of multiple organ dysfunction syndrome, especially concerning the participation of the cardiac tissue in this phenomenon.

Conclusions

The gene response profile associated to oxidative stress has identified eight genes in the cardiac tissue that respond in a hyper-expressive way when the intestine is subjected to ischemia and reperfusion. This study presents the perspective of creating a monitoring protocol in ischemia and reperfusion scenarios from real-time gene expression.

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