The Oxidative Response of Mouse Hearts is Modulated by Genetic Background

Marco Aurélio Santos-Silva[1], Akinori Cardozo Nagato[1], Eduardo Tavares Lima Trajano [1,2], Jackson Nogueira Alves[2], Ana Carla Balthar Bandeira[3], Luís Cristóvão Porto[2], Frank Silva Bezerra[3]

Universidade Severino Sombra[1], Vassouras; Universidade do Estado do Rio de Janeiro[2], Rio de Janeiro, RJ; Universidade Federal de Ouro Preto[3],Ouro Preto, MG, Brazil

Abstract

Background: Smoking plays an important role in cardiovascular diseases. However, the reasons why some individuals develop those diseases and others do not remain to be explained.

Objective: This study aimed at assessing the redox profile of the heart of different mouse strains after exposure to cigarette smoke.

Methods: Male mice of the Swiss (n = 10), C3H (n = 10), BALB/c (n = 10) and C57BL/6 (n = 10) strains were exposed to cigarette smoke (12 cigarettes/day), while their respective controls (n = 10) were exposed to ambient air for 60 days. After being euthanized, their heart was removed for biochemical analyses.

Results: Although the malondialdehyde content did not increase in any of the groups, catalase activity decreased in the Swiss ($p < 0.05$) and BALB/c ($p < 0.05$) strain mice as compared with their respective control groups, while myeloperoxidase decreased in the C3H ($p < 0.05$) and C57BL/6 ($p < 0.001$) strain mice as compared with their respective control groups. The reduced glutathione content decreased in the Swiss, C3H, C57BL/6 ($p < 0.05$) and BALB/c ($p < 0,001$) strain mice as compared with their respective control groups. Regarding reduced glutathione content, an increase was observed in the Swiss strain mice ($p < 0.05$), while a decrease was observed in the C3H ($p < 0.05$) and BALB/c ($p < 0.001$) strain mice as compared with their respective control groups. The reduced glutathione/reduced glutathione ratio showed a reduction in the Swiss and C57BL/6 ($p < 0.05$) strain mice as compared with their respective control groups.

Conclusion: The genetic background of mice can influence the antioxidant response after exposure to cigarette smoke and seems to be a determinant factor for redox imbalance in Swiss and C57BL/6 strain mice. Understanding antioxidant responses and genetic background of C3H and BALB/c strain mice might provide important information regarding cardiac resistance to cigarette smoke. (Arq Bras Cardiol. 2013;100(2):157-163)

Keywords: Oxidative Stress; Tobacco; Smoke; Genetic Enhancement; Mice.

Introduction

Cigarette smoke, a mixture of more than 4,700 substances[1], predisposes to pulmonary[2] and cardiovascular[3] diseases, such as peripheral artery disease and myocardial infarction[4]. The smoking habit increases the risk of those diseases[5]. Cigarette smoke is one of the exogenous sources of free radicals, such as reactive oxygen and nitrogen species (ROS and RNS, respectively). Those oxidants play an important role in the regulation of cell homeostasis, and participate in the pathogenesis of several cardiovascular diseases[6]. The ROSs originating from exposure to cigarette smoke (ECS) have the ability to trigger a chemical reaction chain that leads to irreversible changes, resulting in cell dysfunction and cytotoxicity[7]. To prevent the progression of cellular damage, there is an organized antioxidant and detoxifying system formed by enzymatic antioxidants (superoxide dismutase, catalase and glutathione peroxidase) and non-enzymatic antioxidants (reduced and oxidized glutathione, GSH and GSSG, respectively)[8]. However, in the presence of too much oxidants and/or a deficient protective system, a reduction in catalase (CAT) activity and imbalance between the consumption of GSH and the production of GSSG occur, characterizing oxidative stress[9]. When the reduction-oxidation (redox) system is intact, GSH recovers. The indirect analysis of oxidative stress performed by measuring the activity of antioxidant enzymes, such as CAT, and the concentration of tripeptides, such as GSH and GSSG, and of peroxidases, such as myeloperoxidase (MPO), can provide important information about redox imbalance and future therapies.

Studies have suggested that exposure of different mouse strains to cigarette smoke can impair cutaneous wound healing[10,11]. Organs of different mouse strains respond to oxidative stress induced by cigarette smoke. A study has shown that C57BL/6 and BALB/c mice are the best experimental models to investigate oxidative responses to cigarette smoke

Mailing Address: Frank Silva Bezerra •
Laboratório de Bioquímica Metabólica - Departamento de Ciências Biológicas (DECBI) - Campus Universitário, s/n - Morro do Cruzeiro – Postal Code 35400-000 - Ouro Preto, MG, Brazil
E-mail: frank@iceb.ufop.br, franksbezerra@hotmail.com
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in the liver and lungs, while C3H and C57BL/6 mice are the best models to study those responses in the brain[12]. However, the oxidative responses to ECS in the heart of different mouse strains remain to be clarified. Thus, knowing that might explain why some human smokers develop cardiovascular diseases and others do not. This study aimed at investigating the redox profile of the heart of Swiss, C3H, BALB/c and C57BL/6 mice in response to ECS.

Methods

Animals

Male Swiss, C3H, BALB/c and C57BL/6 mice (20 animals / strain) were stored in groups of ten animals/box at controlled temperature and humidity (21 ± 2ºC, 50% ± 10%, respectively) for eight weeks. They were submitted to inverted 12-hour light/dark cycles (artificial light, 19 h-7 h) and 15 min/h exhaustion cycles at the Department of Histology and Embryology. For each strain, ten animals were exposed to cigarette smoke (ECS groups) and ten animals were exposed to ambient air (control [CTR] groups) for 60 days.

The management and experimentation protocols followed the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (publication 85-23, reviewed in 1985). This project was approved by the Committee of Ethics for laboratory animals (IBRAG – UERJ).

Exposure to cigarette smoke

For 60 days, each animal of the ECS groups was exposed to the smoke of 12 commercial cigarettes per day (three exposures per day), in a chamber for cigarette smoke inhalation. Each cigarette was coupled to a 60-mL syringe, which, after being filled with smoke, was emptied into the inhalation chamber. That procedure ended with each cigarette burning up to its final third, taking, on average, three minutes. Each cigarette produced approximately one liter of smoke, which was diluted into the 30 liters present in the chamber, generating a 3% concentration for inhalation during a total of six minutes per cigarette. After that period, the chamber was opened for total exhaustion of the smoke, and the animals had contact with ambient air for one minute. Then, the procedure was repeated with the remaining cigarettes. During the entire experiment, the animals received a standard food preparation and water ad libitum.

After the 60 days of exposure to cigarette smoke or ambient air, the animals were euthanized by cervical dislocation, and had their hearts removed through a sagittal incision in their chests.

Tissue processing

The heart was placed inside a hemolysis tube with 1 mL of KPE buffer and was homogenized in a tissue homogenizer (Nova Técnica, SP, Brazil), in which the grinding movement was repeated ten times, enabling differential centrifugation. Then, the homogenized samples were centrifuged at 7,500 rpm for ten minutes (FANEM®, SP, Brazil), the supernate was stored at 4 ºC for biochemical analysis, and the pellet was discarded.

Determination of the MPO content

The content of MPO, the enzyme released by neutrophils and that participates in the antioxidant pathway, because it degrades harmful elements, such as hydrogen peroxide, was extracted through the addition of hexadecyltrimethylammonium bromide (HTAB) and 3,3′,5,5′ tetramethylbenzidine (TMB). The tissue (5 to 50 mg) was homogenized with 1 mL of HTAB, or, when the sample originated from body fluids, 100 μl of it were added to 900 μl HTAB in a tube. Then, the samples were centrifuged for 15 minutes at 11,000 rpm (14,000 g) and refrigerated at 4 °C. The supernate was collected and absorbance was determined at 650 nm by using an ELISA plate reader (model 550, Bio-Rad, Hercules, CA, USA).

Analysis of thiobarbituric acid reactive substances (TBARS)

The formation of TBARS during an acid-heating reaction as described by Draper was used as an index of oxidative damage[13]. The samples of homogenized lung were mixed with 1 mL of 10% trichloroacetic acid (TCA) and 1 mL of 0.67% thiobarbituric acid (TBA), and heated for one hour in hot water bath. The TBARS levels were determined by absorbance at 532 nm and expressed as malondialdehyde equivalents (MDA - nM/mg of protein).

Assays of CAT, GSH and GSSG

Catalase activity (U/mg of protein) was measured in response to the amount of H_2O_2 and read at the wave length of 240 nm (14)@[esse número é referência?]@. Aliquots of the homogenate were treated with sulfosalicylic acid at the proportion of 1:1 to remove cellular debris. The supernate was used in the GSH and GSSG assays. The GSH measurement is a cyclic method, because GSH reacts with dithionitrobenzoic acid (DTNB), forming a conjugate (GSH-TNB) and an anion (TNB). Then, the conjugate reacts with the remaining GSH, forming GSSG and TNB, which was measured by spectrophotometry (412 nm). All GSSG present in the sample was converted to GSH through the action of glutathione reductase (GSH-Rd) and NADPH consumption. The TNB production rate is then measured by use of a kinetic method, being proportional to the initial GSH concentration in the sample. GSSG was measured in samples treated with vinylpyridine, to prevent the cyclic conversion of GSH to GSSG. The method to measure GSSG is also kinetic and based on NADPH consumption by glutathione reductase in the reduction reaction of GSSG to GSH. Then, GSH reacts with DTNB, generating TNB, which is then measured by use of spectrophotometry (412 nm)[14].

Statistical analysis

The normality of all data was tested by use of the Kolmogorov-Smirnov test. Data were expressed as mean ± standard error of the mean. Data were analyzed by use of the one-way ANOVA followed by the Bonferroni post-test, in which the significance level adopted was $p < 0.05$. The GraphPad InStat software was used to assess statistical performance (GraphPad InStat, version 3.00 for Windows 95, GraphPad Software Inc.; San Diego, CA, USA).

Results

ECS does not change MDA content in the heart

Aiming at characterizing oxidative stress in our ECS model, we analyzed the oxidative damage by measuring TBARS (MDA). Regarding the MDA content in the different mouse strains exposed to cigarette smoke, no increase in MDA equivalent was observed in the ECS groups as compared with the CTR groups (Figure 1).

ECS reduces CAT and MPO activities in the heart

Regarding the activity of antioxidants in the different mouse strains exposed to cigarette smoke, CAT activity decreased in the Swiss ($p < 0.05$) and BALB/c ($p < 0.05$) groups as compared with their respective CTR groups, while MPO activity decreased in the C3H ($p < 0.05$) and C57BL/6 ($p < 0.001$) groups as compared with their respective CTR groups (Figure 2 A and B).

ECS reduces the GSH/GSSG ratio in the heart of Swiss and C57BL/6 mice

The GSH content decreased in the Swiss, C3H, C57BL/6 ($p < 0.05$) and BALB/c ($p < 0.001$) groups after ECS as compared with their respective CTR groups (Figure 3A). After ECS, an increase in the GSSG content was observed in the Swiss group ($p < 0.05$) and a decrease in the C3H ($p < 0.05$) and BALB/c ($p < 0.001$) groups as compared with their respective CTR groups (figure 3B). The GSH/GSSG ratio decreased in the Swiss and C57BL/6 groups ($p < 0.05$) as compared with their respective CTR groups (Figure 3C).

Discussion

Cardiac remodeling results from geometric and volumetric cardiac changes in response to myocardial injury[15]. Oxidative stress originates from the imbalance between oxidants and antioxidants, and the oxidative damage is caused by that imbalance that modifies cellular macromolecules, leading to cell death due to either apoptosis or necrosis[16]. An experimental study has shown that chronic exposure to carbon monoxide increases the gene expression of endothelin-1 and induces cardiac hypertrophy[17]. Ventricular hypertrophy has been recognized as an important agent in the remodeling process, as previous studies have confirmed that ECS is followed by left ventricular enlargement in rats[18]. Thirty days of ECS are sufficient to impair ventricular function[19]. However, ECS for four months causes ventricular dilation associated with systolic function reduction[20].

Pressure overload causes oxidative stress and cardiovascular system inflammation[3]. The oxidative damage in the cardiovascular system is assessed by use of lipid peroxidation, protein oxidation, and damage to DNA[21]. For over 30 years, TBARS, such as MDA, have been used as important markers of lipid peroxidation[22]. Although C57BL/6 and BALB/c mice have been described as animals prone to lung oxidative damage after a long ECS[12], oxidative parameters following ECS observed in the present study showed a trend towards resistance to cardiac oxidative damage in those strains.

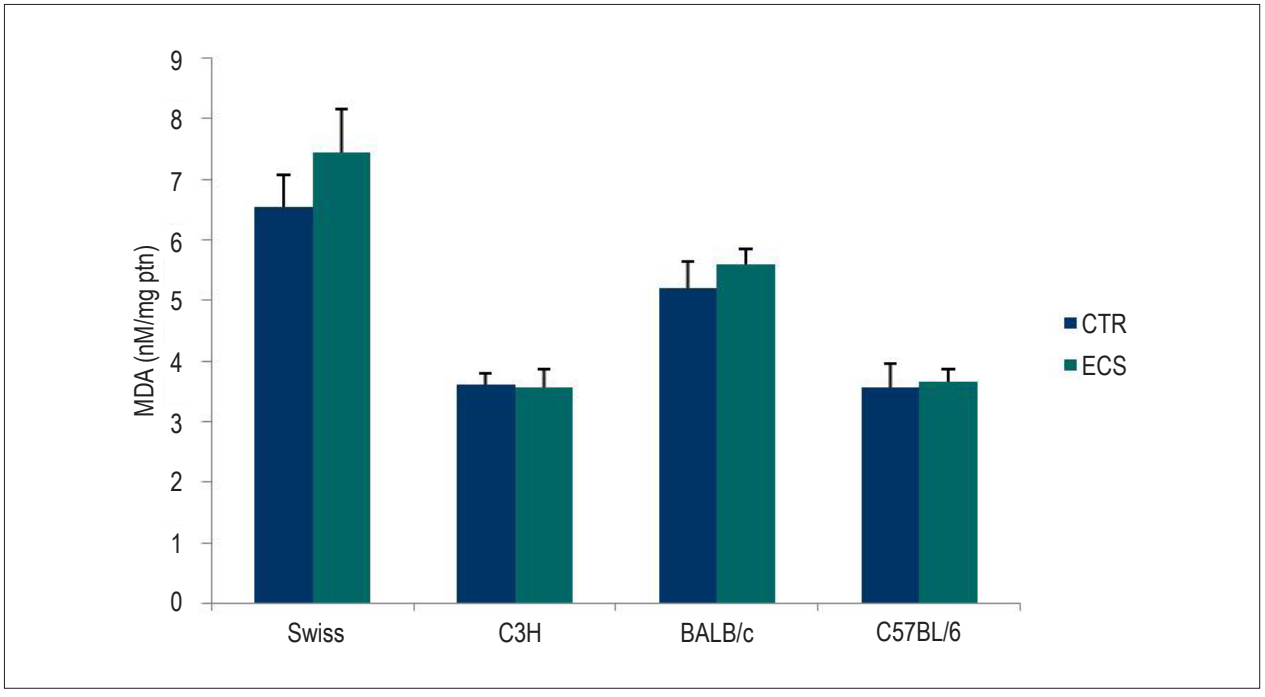

Figure 1 – *Effect of the exposure to cigarette smoke on the concentration of malondialdehyde (MDA) in the heart of different mouse strains. MDA equivalent (nM/mg protein) was measured by use of the thiobarbituric acid reactive substances (TBARS) method. Results of the group exposed to cigarette smoke (ECS) as compared with the control (CTR) group by strain. The values are expressed as mean ± SEM and analyzed by use of one-way ANOVA followed by Bonferroni post-test ($p < 0.05$). *$p < 0.05$; **$p < 0.01$; and ***$p < 0.001$.*

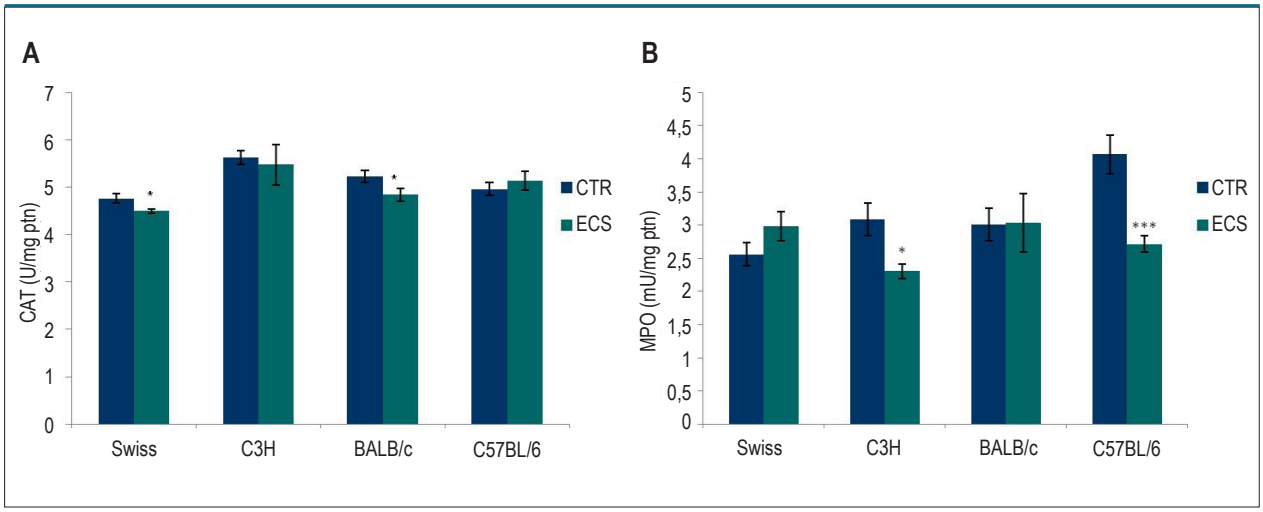

Figure 2 – *Effect of the exposure to cigarette smoke on the catalase (CAT) and myeloperoxidase (MPO) enzymatic activities in the heart of different mouse strains. (A) catalase activity (U/mg protein) and (B) myeloperoxidase activity (mU/mg protein) in the groups exposed to cigarette smoke (ECS) as compared with the control (CTR) group by strain. The values are expressed as mean ± SEM and analyzed by use of one-way ANOVA followed by Bonferroni post-test (p < 0.05). *p < 0.05; **p < 0.01; and ***p < 0.001.*

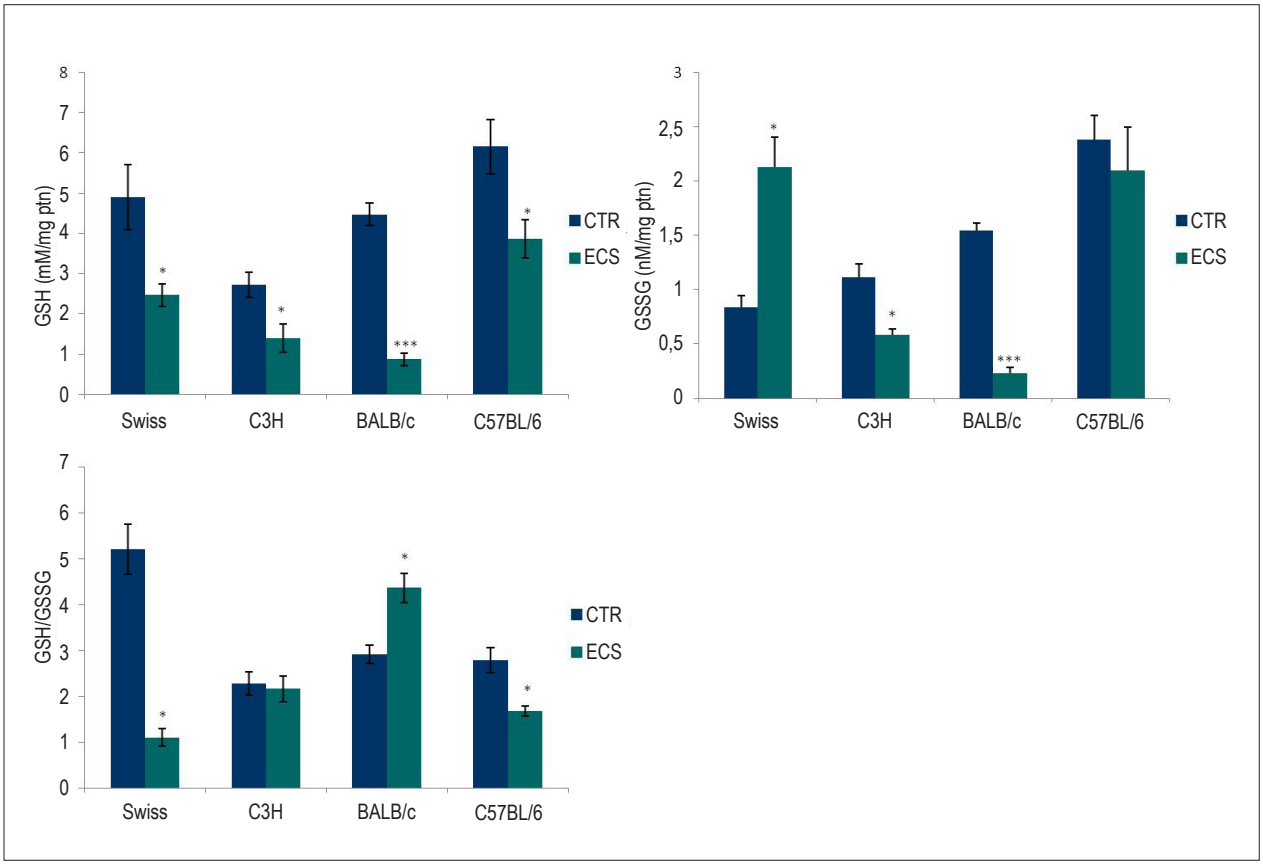

Figure 3 – Effect of the exposure to cigarette smoke on the content of reduced glutathione (GSH) and oxidized glutathione (GSSG) in the heart of different mouse strains. (A) GSH content (nM/mg protein), (B) GSSG content (nM/mg protein), and (C) GSH/GSSG ratio in the groups exposed to cigarette smoke (ECS) as compared with the control (CTR) group by strain. The values are expressed as mean ± SEM and analyzed by use of one-way ANOVA followed by Bonferroni post-test (p < 0.05). *p < 0.05; **p < 0.01; and ***p < 0.001.

Approximately 80% of the studies have found a significant increase in MDA in smokers as compared with non-smokers; however, that result is not unanimous[21-24]. Investigations regarding ECS dose-dependency and MDA content have shown a significant increase according to the daily dose of cigarettes. A study with 298 healthy individuals divided into non-smokers, smokers of less than 30 cigarettes/day, and smokers of more than 30 cigarettes/day has shown a strong relation between ECS and MDA plasma content ($p < 0.0001$)[25]. Another study with 130 healthy volunteers divided into mild smokers (≤ 10 cigarettes/day), moderate smokers (11-20 cigarettes/day), and heavy smokers (> 20 cigarettes/day) has shown that MDA plasma content increased in all groups of smokers as compared with those of non-smokers ($p < 0.05$, $p < 0.001$ and $p < 0.001$, respectively)[26].

Catalase is a cytoplasm hemoprotein that catalyzes the reduction of H_2O_2 to H_2O and O_2[27], being located in the peroxisomes of cardiomyocytes[28]. In transgenic mice, in which there is only catalase isolated from other antioxidants, the enzyme is constitutive and expressed in different amounts in the heart, and, when doxorubicin is administered, catalase activity increases 60 to 100 times, protecting the heart against toxicity[29]. Increased catalase activity causes resistance to ischemia and reperfusion[30]. Our results showed that catalase decreased in the ECS groups of the Swiss and BALB/c strains. BALB/c mice are resistant to cardiovascular diseases induced by an atherosclerotic diet; however, they are sensitive to immune and inflammatory diseases[31].

Nicotine can activate polymorphonuclear cells, inducing neutrophils to produce IL-8. Polymorphonuclear cells significantly contribute to the beginning and progression of vascular inflammation in smokers, in which MPO contributes to the development and progression of cardiovascular diseases[32]. The content of MPO, a neutrophil-derived enzyme that catalyzes the formation of innumerous ROS, is significantly high in smokers as compared with non-smokers[33]. Macrophages use NADPH oxidase to produce O_2^-, which, when undergoing dysmutation, forms H_2O_2; thus, MPO catalyzes reactions with H_2O_2 to generate more potent cytotoxic oxidants, such as hypochlorous acid (HOCl) and the tyrosyl radical, being the only human enzyme that can generate HOCl[34]. Our results have shown a decrease in MPO in the ECS groups of the C3H and C57BL/6 strains. C57BL/6 mice are susceptible to diet-induced lesions. When fed an atherogenic diet (1.25% of cholesterol, 0.5% of cholic acid and 15% of fat) for 14 weeks, they develop atherosclerotic lesions in the aorta[35].

MPO is related to the activation of the protease cascade. The oxidative inactivation of protease inhibitors, such as α-1-antitripsin and tissue inhibitors of metalloproteinases (TIMPs), in association with the activation of proelastases and matrix metalloproteinases (MMPs) affect cardiac remodeling and the stability of atherosclerotic plaques. In a study of MPO null ($MPO^{-/-}$) mice with acute myocardial infarction, the animals showed decreased leukocyte infiltration, reduced ventricular dilation, and preservation of the systolic function[34]. Aldehydes derived from the oxidation of common amino acids, catalyzed by MPO, represent a rapid and relevant mechanism for the generation of cytotoxic species in inflammatory sites[33]. A study has reported an increase in aldehydes in tissues after infarction in wild rats as compared with $MPO^{-/-}$ mice, clearly indicating the role of MPO in the formation of those species[36].

GSH detoxifies chemical agents and eliminates products of lipid peroxidation[37-39]. After GSH exposure to an oxidant, it is oxidized to GSSG. In the inactivation of an oxidant, GSSG is produced and GSH depleted. GSH is recovered by the GSH-Rd enzyme, an essential step to maintain the integrity of the cell protection system[40]. We found a reduction in GSH in all strains of the ECS groups. Our results suggest that GSH-Rd recovered GSH in the C3H and BALB/c strains of the ECS groups as compared with the CTR groups, because a reduction in GSSG was also seen in those groups. However, GSSG in the Swiss strains of the ECS groups remained increased. When the redox system is intact, GSH recovers. Our results showed that the GSH/GSSG ratio decreased in the Swiss and C57BL/6 strains of the ECS groups, indicating that both strains developed oxidative stress.

Conclusions

Our results show that oxidative stress should be considered in different populations with cardiovascular disease, and start a new discussion about the relationship between the genetic profile and redox imbalance in experimental models of ECS.

Author contributions

Conception and design of the research: Santos-Silva MA, Nagato AC, Bezerra FS; Acquisition of data: Santos-Silva MA, Nagato AC, Trajano ETL, Alves JN; Analysis and interpretation of the data: Santos-Silva MA, Nagato AC, Trajano ETL, Alves JN, Bandeira ACB, Bezerra FS; Statistical analysis: Bandeira ACB, Porto LC, Bezerra FS; Obtaining financing: Porto LC; Writing of the manuscript: Trajano ETL, Alves JN, Bandeira ACB, Porto LC, Bezerra FS; Critical revision of the manuscript for intellectual content: Porto LC, Bezerra FS.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

This study is not associated with any post-graduation program.

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