Myeloperoxidase as an Indicator of Oxidative Stress in Metabolic Syndrome

Mieloperoxidasa como indicador de estrés oxidativo en síndrome metabólico

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ABSTRACT

Background: Increased myeloperoxidase (MPO) activity would be the link between the rise of the inflammatory response and oxidative stress (OS) in metabolic syndrome (MS).

Objective: The aim of this study was to determine the enzymatic activity of MPO associated with OS in animals with MS and establish their relationship with probable cardiovascular injury.

Methods: Male Wistar rats were divided into two groups: Group A, control (n=12) and Group B, induced MS (n=12). Metabolic syndrome was produced by 6-week administration of 10% fructose diluted in the drinking water. Insulin (μU/ml), glucose (mg/dl), lipid panel (mg/dl), HOMA (homeostatic model assessment), MPO (IU/ml) and superoxide dismutase (SOD) activity (U/ml) were measured. Light microscopy was used for the histological study of the heart and thoracic aorta.

Results: Group B showed significantly increased levels of plasma glucose (176±17.3 mg/dl), insulin (29.5±4.52 μU/ml), HOMA (11±1.3), total cholesterol (133±9.6 mg/dl) and triglycerides (75±12.9 mg/dl) compared with Group A: plasma glucose (115±1.1 mg/dl), insulin (4±0.82 μU/ml), HOMA (3±0.38), total cholesterol (69.7±1.6 mg/dl) and triglycerides (46.2±6 mg/dl), (p<0.001 for all variables). A significant decrease in HDL (28.3±1.14 mg/dl) in Group B vs. Group A (61±1.0 mg/dl) (p<0.001) validated the experimental MS model. Myeloperoxidase activity increased significantly in Group B (181.3±15.7 IU/ml) vs. Group A (116.07±4.2 IU/ml) (p<0.001). A similar behavior was seen with SOD antioxidant activity in Group B (181±6 U/ml) vs. Group A (138±3.6 U/ml) (p<0.01). Light microscopy of the heart and thoracic aorta revealed histopathological changes in animals with induced MS.

Conclusion: Increased MPO and SOD in Group B would indicate the presence of OS in MS, with consequences at the vascular level.

Key words: Myeloperoxidase - Oxidative Stress - Metabolic Syndrome - Insulin Resistance - Cardiovascular Risk

RESUMEN

Introducción: Los incrementos en la actividad de la mieloperoxidasa (MPO) serían el nexo entre el aumento de la respuesta inflamatoria y el estrés oxidativo (EO) en el síndrome metabólico (SM).

Objetivo: Determinar la actividad de la enzima MPO asociada con EO en animales con SM y establecer su relación con las probabilidades de lesiones cardiovasculares.

Material y métodos: Se utilizaron ratas macho de la cepa Wistar, que se dividieron en: Grupo A, control (n = 12) y Grupo B, inducción de SM (n = 12). El SM se indujo con la administración de fructosa al 10% diluida en agua de bebida durante 6 semanas. Se cuantificaron insulina (μU/ml), glucemia (mg/dl), perfil lipídico (mg/dl), HOMA (homeostasis model assessment), MPO (UI/ml) y actividad de la superóxido dismutasa (SOD) (U/ml). Se estudió la histología de la aorta torácica y el corazón por microscopía óptica.

Resultados: Se observaron niveles de glucemia (176 ± 17,3 mg/dl), insulina (29,5 ± 4.52 μU/ml), HOMA (11 ± 1,3), colesterol total (133 ± 9.6 mg/dl) y triglicéridos (75 ± 12.9 mg/dl) incrementados significativamente en el Grupo B en comparación con el Grupo A: glucemia (115 ± 1,1 mg/dl), insulina (4 ± 0,82 μU/ml), HOMA (3 ± 0,38), colesterol total (69,7 ± 1,6 mg/dl) y triglicéridos (46,2 ± 6 mg/dl) (p< 0,001 para todas las variables); se verificó disminución significativa de los valores de HDL (28,3 ± 1,14 mg/dl) en el Grupo B en comparación con el Grupo A (61 ± 1,0 mg/dl) (p< 0,001), validando así el modelo experimental de SM. La actividad de la MPO se incrementó significativamente en el Grupo B (181,3 ± 15,7 UI/ml) respecto del Grupo A (116,07 ± 4,2 UI/ml) (p< 0,001). Similar comportamiento presentó la actividad antioxidante de la SOD en el Grupo B (181 ± 6 U/ml) respecto del Grupo A (138 ± 3.6 U/ml) (p< 0,01). Las microscopías ópticas de corazón y de aorta torácica evidencian cambios histopatológicos en los animales con SM inducido.

Conclusión: Los incrementos de la MPO y la SOD en el Grupo B demostrarían la presencia de EO, con repercusión a nivel vascular en el SM.

Palabras clave: Mieloperoxidasa - Estrés oxidativo - Síndrome metabólico - Insulinorresistencia - Riesgo cardiovascular


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INTRODUCTION

Oxidative stress (OS) is currently considered a potential cause of inflammation, implicated in the development of chronic systemic diseases, as metabolic syndrome (MS). The presence of OS implies an imbalance in oxidation-reduction metabolism generated by the uncontrolled production of reactive oxygen (ROS) and nitrogen species (RNS). (1, 2) It is known that overproduction of ROS and RNS in the vascular wall generates endothelial dysfunction, a condition that increases the risk of developing cardiovascular diseases (CVD).

The prooxidative state triggered by OS may induce insulin resistance through the phosphorylation of insulin receptors and increase in proinflammatory cytokine levels, both conditions expressed in MS. (3) Moreover, it has been postulated that excessive formation of reactive species would directly impact on insulin effect, modifying antioxidant enzymatic mechanisms such as superoxide dismutase (SOD). Superoxide dismutase catalyzes the breakdown of harmful oxidants, neutralizing their toxicity and avoiding their pathological concentrations; (4) thus, OS would condition endothelial dysfunction. (5)

In the continuous search of new prooxidant markers, myeloperoxidase (MPO) acquires relevance to identify the factors determining MS. (6) Excessive MPO activity may lead to tissue injury through the production of oxidants, thus forming lipid and protein reactive species. Recent studies have shown that exposure of activated leukocytes to low density lipoproteins (LDL) generates, through MPO, nitrogen and halogenated species facilitating lipid peroxidation, protein nitration and conversion to proatherogenic forms of LDL in the vascular wall. (7) In addition, MPO participates in the generation of dysfunctional high-density lipoproteins (HDL) transforming their anti-inflammatory properties to proinflammatory ones. (8)

Also, proinflammatory and prooxidative states have been implicated in myocardial damage and endothelial impairment, (9) which emphasizes the importance of implementing oxidative biomarkers, as MPO, in asymptomatic stages of MS. This enzyme could define cardiovascular risk and would be an indicative test for this pathology. (10)

The purpose of this study was to determine MPO activity associated with OS in animals with MS and establish its relationship with probable cardiovascular injury.

METHODS

Animals
Male Wistar rats, weighing 280±20 g, inbred in the Institute of Physiology of the School of Medicine at Universidad Nacional de Córdoba were used to perform the experimental and analytical study. Animals were fed a balanced rat diet containing at least 17% protein.

Study groups
Twenty-four male rats were divided into two groups and consecutively studied in either of two different experimental situations:
- Group A: Control, animals without intervention (n=12)
- Group B: MS, induced by hydric diet supplemented with 10% fructose in the drinking water for 6 weeks (n=12).

Induced metabolic syndrome
Metabolic syndrome was induced by the administration of 10% fructose diluted in the drinking water for 6 weeks. (11) To confirm the presence of MS, fasting blood glucose, insulin, triglycerides, total cholesterol and HDL cholesterol were determined and HOMA (homeostatic model assessment) was calculated.

Biochemical assays

Plasma samples
Blood was obtained from decapitated animals previously anesthetized with ketamine (Ketal®) (16.25 mg/kg) and 2% xylazine (Alfasan®) (2 mg/kg), 42 days after fructose administration or tap water and following 24-hour fasting. Blood was collected in Petri dishes with an anticoagulant combination of ammonium/potassium oxalate in a 2:1 ratio. EDTA-anticoagulated blood samples were used for SOD and MPO assays. Then, blood was centrifuged at 3000 rpm for 15 minutes to obtain plasma and lysed red blood cells, respectively.

The following variables were quantified
- Glucose: Plasma glucose levels were determined by spectrophotometry, using commercial kits (Wiener, Buenos Aires, Argentina) according to the enzymatic method. Results were expressed in mg/dl. (12)
- Insulinemia: Insulin levels were quantified by radioimmunoassay and the results were expressed in μU/ml. (13)
- The homeostasis assessment model (HOMA) was used as index to measure the degree of insulin resistance and was calculated using the formula: [insulin (μU/ml) × plasma glucose (mmol/L)]/22.5. (14)
- Lipid panel: The lipid panel was assessed by enzymatic method (15) and the results were expressed in mg/dl.
- Myeloperoxidase: ELISA was used to quantify MPO enzymatic activity, and the results were expressed in IU/ml. (16)
• Superoxide dismutase: SOD enzymatic activity was assessed by spectrophotometry in lysed red blood cell using the Randox kit. Results were expressed in U/ml. (17)

Tissue samples for pathological anatomy by light microscopy
In all animals, 16 heart samples were collected, performing 4 μm longitudinal sections, and 30 thoracic aortic samples were collected from its origin to the last thoracic portion performing 4 μm sections. Samples for pathological anatomy were fixed in 10% buffered formalin and stained with hematoxylin-eosin. Light microscopy analysis was performed at 40×, 60× and 400× amplification

Statistical analysis
Multivariate ANOVA followed by the Hotelling post-hoc test was used to compare all possible combinations of pairs of means. In all cases, the significance level was set at p<0.05.

Ethical considerations
The procedures were performed following the guidelines and protocols approved by the Institutional Committee for the Care and Use of Laboratory Animals of the School of Medicine at Universidad Nacional de Córdoba, based on the American Physiological Society “Principles for the Care and Use of Laboratory Animals” (NIH Publication 85-23, 1996).

RESULTS
Biochemical characteristics to validate the MS model in the study groups are shown in Table 1.

Blood glucose, insulin, increased total cholesterol and triglyceride levels together with elevated HOMA and decreased HDL values in group B (MS) validate the experimental model (see Table 1).

Assessment of MPO activity evidenced a statistically significant increase in the group with induced MS (181.3±15.7 U/ml) vs. the control group (116.07±4.2 U/ml) (p<0.001). This increased MPO activity would indicate the proinflammatory state of the animals with MS, as illustrated in Figure 1.

Results of SOD enzymatic activity in lysed red blood cells of rats with MS and control rats are shown in Figure 2.

The persistence of the proinflammatory and prooxidative stimulus in group B (MS) (181±6 U/ml) generated a significant increase in the enzymatic activity of SOD when compared with group A (control) (138±3.6 U/ml) (p<0.01).

The increase in MPO and SOD in animals with experimentally induced MS compared with the control group would indicate the persistence of the proinflammatory and prooxidative stimulus in the group with MS.

Figures 3 and 4 show the vascular and cardiac impact in rats with experimentally induced MS.

DISCUSSION
The experimental model of MS showed that rats chronically receiving fructose in the drinking water provide a useful model for the diagnosis of factors constituting MS, which is induced by changes in food intake and expresses numerous alterations similarly to humans with MS. (18) The administration of 10% fructose in group B (MS) generated hyperglycemia, hypertriglyceridemia, reduced HDL levels and increased total cholesterol levels, together with hyperinsulinemia, confirming that the experimental model presents characteristic manifestations of MS. In addition, the assessment of blood glucose/insulin homeostasis, HOMA parameter, constitutes a useful model.

<table>
<thead>
<tr>
<th>Table 1. Plasma levels of glucose, insulin and lipid panel in group A (control) and group B (metabolic syndrome)</th>
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<tbody>
<tr>
<td>Control (A)</td>
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<tr>
<td>Blood glucose (mg/dl)</td>
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<tr>
<td>Insulinemia (μU/ml)</td>
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<td>HDL (mg/dl)</td>
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<td>Triglycerides (mg/dl)</td>
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<td>HOMA</td>
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Results are expressed as mean±standard error of the mean. n = 12. * p <0.001 vs. control rats (A). MS: Metabolic syndrome. HDL: High density lipoproteins. HOMA: Homoeostatic model assessment.

Fig. 1. Quantification of myeloperoxidase (MPO) activity in control (A) and with metabolic syndrome (MS) (B) rats. Values are expressed as mean±standard error of the mean. n = 12. * p <0.001 vs. control rats (A).

Fig. 2. Analysis of superoxide dismutase (SOD) enzymatic activity in control and with metabolic syndrome (MS) (B) rats. Values are expressed as mean±standard error of the mean. n = 12. # p <0.001 vs. control rats (A).
to quantify insulin resistance, reflecting beta cell function, only needing a fasting serum sample. Our results showed an increased HOMA value in the MS group, indicating the presence of insulin resistance.

Myeloperoxidase is an important cardiovascular risk factor, producing an increase in the inflammatory response capable of enhancing the oxidative effects of its cosubstrate hydrogen peroxide (H$_2$O$_2$), with a main role in endothelial damage and inflammation. (19)

Several investigations describe the association of chronic inflammatory states and insulin resistance with increased MPO activity, (20) increased OS, endothelial dysfunction and their influence on cardiovascular risk. (21) In our work, the significant positive relationship between fasting glucose/insulin (HOMA) and MPO activity, confirms the probable participation of dysglycemia in the altered redox metabolism of animals with MS.

Increase SOD activity associated with modifications in fasting blood glucose levels, insulin resistance, dyslipidemia due to decreased HDL and hypertriglyceridermia, and increased proinflammatory and prooxidative components are indicators of OS present in the experimental model of MS. This situation reflects the importance of SOD as endogenous antioxidant mechanism attempting to compensate the increase in free radicals, though it is not always enough, either by its low productivity or because it is overcome by the enzymatic production rate. (22-24) Concurrent with the biochemical parameters analyzed in this experimental model, thoracic aorta histopathological results with light microscopy show the vascular impact of this disease. (25)

The proinflammatory and prooxidative signals triggered by the biomarkers present in the experimental model of MS probably activate the endothelium using the superoxide radical to generate the free radical chain reaction initiating lipid peroxidation, causing loss of cellular structure and function as observed in the histopathology of the heart and thoracic aorta. (26) Several authors have established the combination of proinflammatory state, insulin resistance and obesity with the progression of endothelial dysfunction, (27-29) which is a common factor for the development of MS and CVD.

The results of this study demonstrate that insulin resistance evidenced by hyperinsulinemia, modified HOMA, decreased HDL levels and hypertriglyceridermia are associated with the proinflammatory properties and endothelial dysfunction demonstrated in the experimental model of MS.

CONCLUSION

Increased MPO and SOD enzymatic activity in the group with induced MS, together with the anatomo-pathological findings would demonstrate increased OS and would justify the usefulness of these markers for the evaluation of cardiovascular risk in the early stages of MS.

Conflicts of interest

None declared. (See authors’ conflicts of interest forms in the website/Supplementary material).

REFERENCES


