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Involvement of Oxidative Stress and Nitric Oxide in Fibromyalgia Pathophysiology: A Relationship to be Elucidated

Andrei Pereira Pernambuco[1,2,3*], **Luana Pereira Leite Schetino**[3,4], **Luciana de Souza Cota Carvalho**[3,5] **and Débora d'Ávila Reis**[3]

[1]*University Center Formiga, MG. Avenida Doutor Arnaldo Senna , 328. Red Water - Formiga, Minas Gerais, Brazil*

[2]*University of Itauna, MG. MG highway , 431 Km 45 , s / n - Green Campus, Itauna , Minas Gerais, Brazil*

[3]*Institute of Biological Sciences, Federal University of Minas Gerais. Avenida Presidente Antônio Carlos , 6627 - Pampulha , Belo Horizonte , Minas Gerais, Brazil*

[4]*Federal University of the Jequitinhonha Valley and Mucuri . Campus I - Diamantina , Minas Gerais, Brazil*

[5]*Hospital Mater Dei. Avenida do Contorno, 9000 - Barro Preto, Belo Horizonte - Minas Gerais, Brazil*

[*]**Corresponding author:** Andrei Pereira Pernambuco, Rua Araxá – 206, Residencial Morro do Sol, Itaúna, Minas Gerais, Brazil, Tel: +55 (37) 3242-1477; E-mail: pernambucoap@ymail.com

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Abstract

Fibromyalgia (FM) is a rheumatic syndrome characterized by chronic widespread pain that is often associated with other signs and symptoms such as sleep disturbances, headache, fatigue and morning stiffness. To date, the pathogenesis of fibromyalgia is unknown, several studies have been conducted in or to clarify it. Genetic and psychological abnormalities, dysfunctions of the central nervous system, endocrine and immunological disorders as well as abnormalities of the peripheral microcirculation have been identified in FM patients, demonstrating that many factors may interact in the pathogenesis of this condition. Recent research suggest that oxidative stress (OS) and nitric oxide (NO) may be directly involved in the pathophysiology of this syndrome, however, the mechanism by which such interference takes place is not fully elucidated. Currently, there is an increasing number of research papers that demonstrate both, altered concentrations of oxidants resulting from the OS mechanism, and antioxidants as for example NO among patients with FM. Thus, the purpose of this review was to gather and organize part of the information available about the participation of NO in the OS in the symptoms of FM.

Keywords: Fibromyalgia; Nitric oxide; Oxidative stress

Introduction

The fibromyalgia (FM) is a rheumatologic syndrome characterized by chronic pain associated to others signs and symptoms, such as, sleep disturbances, fatigue, depression, anxiety and others [1]. Until now, its pathophysiology remains unclear and seems to be the result of multiple interactions of endogenous and exogenous factors [2-6]. Many studies have proposed that the nitric oxide (NO) and oxidative stress have an important role in the genesis and evolution of FM [7-11].

The NO is a gas that comes from the catalysis of L-argine by dioxygenase. It has a great diffusion capacity between the body tissues that's allows its participation in the regulation of many different process, such as, nociception modulation, vascular tonus regulation, immune function, neurotransmission and excitation-contraction coupling [12,13]. The NO has yet a role as an antioxidant, preventing the accumulation of reactive oxygen species (ROS) inside the cell avoiding the occurrence of oxidative stress [13-15]. In the current literature the vast majority of studies confirm the relation of oxidative stress and NO in FM pathophysiology [7-11], but some others, mainly those that evaluate the NO failed to confirm such interaction [16-19]. Unfortunately none of them could decode completely this interaction [10,11,15].

In this way, the main goal of this review is to gather and organize part of the available literature about the role of NO and oxidative stress in the FM pathophysiology to contribute for a better comprehension of this complex interaction.

Fibromyalgia

The FM is a widespread musculoskeletal chronic pain (more than three months) that involve the body above and below the belt line, the right and left side of the body and at least one component of the axial skeleton [20]. Many others non painful symptoms are generally present and contribute to the clinical condition, such as, fatigue, depression, sleep disturbances, stiffness, irritable bowel syndrome, cystitis and others [1]. The classical flogistic signs are absent and the routine laboratory tests are usually normal [6,21].

The first diagnostic criteria were defined in 1990 by the American College of Rheumatology and they determine FM as a widespread chronic pain with at least 11 of 18 tender points established [20]. In 2011, the American College of Rheumatology modified the previous criteria to reach the others symptoms frequently associated to this condition [1]. They also exclude the tender points count and include a widespread pain index and a severity scale [1].

It is estimated that FM affects approximately 5% of the world population, making it one of the most common rheumatic diseases worldwide [22]. Women are more affected than men, by a ratio of up to nine cases in women for every case diagnosed in men [6].

Until now, no treatment is completely efficient, whether pharmacological or non-pharmacological which prompts to persistence of symptoms [5,6]. The chronicity and intensity of symptoms act negatively in the quality of life and labor capacity of the patients [23,24].

The etiology remains unclear, but seems to be a result of complex interaction of multiple factors, such as, genetic, neuroendocrine, immune, psychosocial, environmental and behavioral [2-6]. In addition to a possible involvement of NO and oxidative stress as showed by some authors [7-11].

Oxidative Stress

Most ROS are generated as by-products during mitochondrial electron transport. They are chemical elements highly reactive that had an unpaired electron and are formed by the catalytic transition of heavy metals, such as, iron, copper or manganese [19]. Its main source in the body comes from oxygen then every radical that forms from oxygen are named ROS [25]. High levels of ROS has been associated to some neuropsychiatry and rheumatology diseases, such as, Alzheimer disease, depression [15,26], rheumatoid arthritis, ankylosing spondylitis and chronic fatigue syndrome [27,28].

Under normal physiological conditions the ROS production is highly regulated because its balance with antioxidants is extremely important for the cell homeostasis [7,9]. The antioxidants are responsible for remove the free radicals but sometimes they are suppressed by the overproduction of them [13,22]. This implies in ROS accumulation, which in turn attack the polyunsaturated fatty acids present in the lipid bilayer of the cell membrane, eventually leading to lipid peroxidation (LPO) resulting in changes in the resting potential of the membrane, loss of its fluidity property and causing sometimes its rupture with consequent release of the cell content [29-31]. The LPO leading production of toxic and reactive aldehyde metabolites such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE). Those metabolites are extremely toxic and can be release between tissues causing the oxidative damage [30].

The nerve and muscle are the more sensitive tissues to oxidative damage, since they are higher oxygen consumers, have high concentrations of fatty acids and relatively low concentrations of enzymatic and not enzymatic antioxidants [13,19,32]. In this context, some studies have shown the presence of damage to muscles and or neurons induced by ROS [13,33]. These lesions occur, probably due to the degradation of polyunsaturated fatty acids of the membrane, as well as the reduction of the superoxide dismutase activity (SOD), the major intracellular anti-oxidant enzyme, and total antioxidant capacity, strongly suggesting a condition of oxidative stress [7,13,33]. The studies that seek to evaluate the levels of lipid peroxidation often use markup substances reactive to thiobarbituric acid, MDA or HNE [29]. However, tests involving MDA are currently the most accomplished, since they can be used to assess lipid peroxidation in plasma, serum or lysed cells [15].

The use of antioxidants in neuromusculoskeletal conditions, including FM has gained scientific backing, due to good results in symptom relief. The antioxidant supplementation restores the balance between ROS and antioxidant thereby protecting tissues from harmful effects of the oxidative stress [9,11,18,19,22]. In this context, Cordero et al., showed that CoQ10 supplementation was able to affect both subjective and objective variables, such as, Fibromyalgia Impact Questionnaire (FIQ), pain, fatigue, anxiety, depression, morning stiffness) as well as improving the expression of SOD related genes, the mitochondrial biogenesis and modulating genes related to the production of pro-inflammatory cytokines such as IL-6, IL-8 and TNF in FM patients [22]. Miyamae et al., demonstrated that supplementation with ubiquinol-10 was able to increase the levels of CoQ10 and reduce the levels of free cholesterol, cholesterol esters and fatigue in FM patients [9].

Nitric Oxide

The nitric oxide is a thermodynamically unstable gas with the highest diffusion coefficient of any biological molecule. This property makes it one of most important molecule in the control of some cellular and organ functions [13]. The NO is involved in many biological functions, such as, modulation of nociception, vascular tone, bronchodilation and immune function [13,33,34]. The NO is synthesized by the enzyme nitric oxide synthase (NOS). This enzyme has three isoforms: neuronal (nNOS), inducible (iNOS) and endothelial (eNOS), all of them using the amino acid L-arginine as precursor of NO [13,14]. The nNOS and eNOS are also known as constitutive (cNOS) because they are constitutively expressed in the majority of the cells while iNOS is expressed only in response to some pathological stimuli. The cNOS are regulated by Ca2+ fluxes and subsequent binding of calmodulin, while iNOS is regulated by cytokines, cell to cell contact and environmental pH [34,35].

The NO has been proved to participate in many immune activities, such as, intercellular signaling, chemotaxis and adhesion of leukocytes, selection and development of T-cells in thymus, inhibition of tumor cell growth, induction of tumor cell death by activated macrophages, infection control, inflammation, autoimmunity and graft rejection [12,14,36-42]. In addition to others activities, such as, modulation of nociception, control of vascular tone, control of some neurotransmission in both central and peripheral nervous system [13,14,33,43]. This diversity of function is due in large part to the fact that NO interact with any other inorganic molecule (such as oxygen and superoxide), DNA structures (pyridimine bases), protein groups (heme) and proteins (interacting with tyrosine or thiol groups) [44]. It is possible to understand the notable pleiotropism of NO if we considered the fact that its main target are auto regulated molecules as example transcription factors and intracellular signaling cascades components [45].

Direct measurement of NO in the biological samples is very difficult because it's unstable properties. Therefore, the stable oxidation end products of NO, NO_2- and NO_3- can be readily measured in biological fluids and have been used *in vitro* and *in vivo* as indicators of NO production and have been used to clarify the role of NO in many pathological conditions [13,18]. Another indirect measurement of NO concentration is measure their enzyme NOS by immunoassay [19].

The role of NO and oxidative stress in the FM syndrome

The studies that evaluate the NO concentrations in FM are very contradictory [7-15]. There are evidences that point to absent of NO alterations [16-19], while others advocate decreased levels of NO [13,46] or even increased levels of NO in FM patients [47-49]. This conflict of evidences can be explained by the fact that the NO production can be sourced of cNOS or iNOS, then alteration in any of these forms could raise de NO level, but not necessarily represents a pathological condition [50].

Although the NO has great antioxidant property, in some pathological conditions it could contribute to development and aggravate the clinical condition, as for example, in septic shock, autoimmunity diseases, stroke and diabetes mellitus [51]. Furthermore, the NO may play a beneficial or deleterious way depending on their concentrations, and some of their deleterious

effects are related to protein nitration [52]. Differently from NO studies, the studies that evaluate the role of oxidative stress in FM are very robust and they advocate in favor of the oxidative stress in the FM pathophysiology [7-11,15,28]. There are evidences based on serum samples [7,18], plasma [7,9,15,53], blood mononuclear cells (BMC) [7,15] and skin biopsy [8]. According to recent studies, the antioxidant activity in FM patients is significantly reduced [8,53] because the decreased of both enzymatic and non-enzymatic antioxidants [7,8]. Low levels of antioxidants are unable to oppose the ROS, causing their accumulation inside the cells and then the lipid peroxidation which results in the destruction of polyunsaturated fatty acids of the cell membrane and cell death [15].

It's important to mention that lipid peroxidation is direct related to the score of fibromyalgia impact questionnaire (FIQ) and some FM symptoms, such as, pain and depression [7,15]. And it is the probable relationship between oxidative stress and the inflammatory process in FM patients [8,22]. Despite all the evidence, it is difficult to confirm whether the oxidative stress is cause or consequence of FM [11]. In order to clarify those questions, it will be discussed below separately the relationship of pain, fatigue and inflammation with NO and oxidative stress in FM patients.

Relationship of NO and oxidative stress with pain in FM

It's known that most FM patients present the central sensitization phenomenon which consists in the exacerbation of neuronal responsiveness, particularly to noxious stimuli [54]. This phenomenon happens after repeated and frequent noxious stimuli which in turn leads to a rearrangement of the plasticity of central nervous system [55,56]. This new status cause chemical and electrophysiological changes that result in exacerbated response of painful stimuli (hyperalgesia) and or increased perception of non-noxious stimuli that is wrong perceived as pain (allodynia) [55,56]. The central sensitization may involve the generation of referred pain and hyperalgesia through the involvement of multiple spinal segments [55,57]. The rise of substance P inside de dorsal horn of spinal cord seems to be the main alteration that causes the central sensitization. After all, substance P is one of the major neurotransmitters involved in the modulation of pain response and it's responsible to enhance the conduction of stimuli to supraspinal center of pain, such as, the thalamus, the insula and somatosensory cortex [58].

Experimental studies have shown that NO is also an important neurotransmitter involved in the spinal pain pathway and that the sensitization of those pathways can occur or be related to NOS activation with subsequent rise of NO [59]. In addition, the NO cause the production of cyclic guanosine monophosphate (cGMP) in the nearest neurons, and depending on cGMP expression, the NO may act to inhibit or excite the target neurons [59,60]. Therefore, the NO can actively participate in the hyper sensitization in FM since it is able to influence the rise of neurotransmitters, as example, substance P in the central terminal [59]. This process seems to depend on activation of N-methyl-D-aspartate (NMDA) receptors which are stimulated by C-fiber and while opened allows the calcium influx stimulating the NOS to synthetize the NO [60]. One study confirmed the NO involvement in the central sensitization in spinothalamic tract of primates that may suggest a reciprocal NO actions in humans [61].

Another important point is the interaction between NOS and cyclooxygenase 2 (COX-2) in central nervous system. This relationship has not been fully elucidated, but its known that both enzymes takes part of hyperalgesia and allodynia caused by NMDA stimulation [62]. As NOS and COX-2 acting quite closely, it is possible that NO stimulates COX-2 by the binding of heme to the active site of COX-2 enzyme [62]. This hypothesis is reinforced by studies that use the NOS or COX-2 inhibitors to relief chronic pain [63,64]. The NOS inhibitors were also used in chronic headache with satisfactory results [65]. The results of these and other studies suggest that the use of NOS inhibitors can be a very plausible alternative for the treatment of painful symptoms such as those presented in FM syndrome [47].

On the other hand, the lipid peroxidation, one of the main consequences of oxidative stress, can interfere in the etiology of pain perception, either by induction of central or peripheral hyperalgesia. The accumulate of superoxide inside the cells stimulate the hyperalgesia and change the nociception by the release of pro-inflammatory cytokines (such as IL -1 , IL-6 and TNF), peroxynitrite and activation of polymerase [66]. Although the mechanisms by which oxidative stress can alter muscle sensitivity are not completely understood, it is believed that they should, at least in part, to the fact that oxidative damage can cause a local reduction of nociceptors and thereby reduce the pain threshold [67].

Relationship of NO and oxidative stress with fatigue in FM

There are many hypotheses to explain the fatigue in FM. The most acceptable is related to disturbances in the peripheral microcirculation [68]. According to that, the changes in the physiological flux cause an imbalance between demand and supply of oxygen changing many body functions [69]. Apparently, these abnormalities are initiated after a decrease in endothelial NO production, resulting in microcirculatory disturbances which in turn hinder the oxygenation of certain regions generating muscle tissue ischemia foci [46]. Those foci interfere negatively in the production of muscle strength [70], and significantly increase the recovery time after the physical exercises [71]. Since the NOS is a mediator of the reduction process of two electrons in the O_2 to generate the H_2O_2 [72], the decreased of NO may result in inadequate synthesis of ATP by the mitochondria causing tissue acidosis [71]. The decrease of NO also result in an imbalance between oxidants and antioxidants that's impair the cell function [46,72]. This imbalance has been shown in FM patients [7,28,72,73].

Another important role of NO that can reduce the fatigue is to stimulate the mitochondrial biogenesis [74,75]. In the skeletal muscle the rise of mitochondria can increase their performance due to more efficient use of energy [76]. And assuming that some FM patients have reduced levels of NO [13,46], it becomes even more important to implement activities that tend to increase NO levels in these patient, such as, aerobic exercise to alleviate the fatigue [77].

In this context, it should be noted that oxidative stress and mitochondrial damage are common events in FM patients [8,28] which impact on the energy production, since the main role of mitochondria is provide energy to the cell and organelles [8,9,28,78]. Interestingly, the superoxide, a major ROS found in the body, is released from the mitochondria complexes I and III of the electron transport chain [78]. Furthermore, it has been shown in FM patients a decrease in mitochondrial activity, particularly by reduced levels of activity of complexes I, II , III and IV of the electron transport chain [8], and mitochondrial enzyme activity dependent of CoQ as well as the reduction of intracellular ATP [22,28]. All together may explain the fatigue and exercise intolerance witnessed by FM patients. Important to mention that supplementation with CoQ10 or ubiquinol was able to significantly improve fatigue in FM patients [9,22].

Relationship of NO and oxidative stress with inflammation in FM

Many studies have been performed in order to evaluate the etiologic role of the immune system in FM, specifically regarding the presence or absence of inflammation in these patients [79-84]. The literature is very contradictory regarding the levels of inflammatory cytokines in FM. This may be due to the following factors:

- Different methods for measuring cytokines
- Evaluation of women in the period pre or post menopause
- Differences in body mass index - BMI
- Measure of serum or plasma levels of cytokines that does not reflect accurately the expression or release of cytokines in and by circulating cells or tissues
- Use of medicines
- Time of collection of the sample, because the cytokine concentrations may vary at different times of day.

Recently, it has been suggested that cytokine gene expression in relevant tissues may be a way to improve the accuracy of measurements [85]. In FM, there are two studies that evaluate the tissue expression of cytokine. One of them uses the painful point in the vastus lateralis of the quadriceps muscle [86] and the other skin biopsy [8]. In the first study, it was measure the expression IL-1β, IL-6, IL-8, and TNF in three samples: before, during and after 20 minutes of dynamic repeated contractions, but it was not found any difference between FM patients and control group and none correlation between cytokines, pain and fatigue [86]. In the second study, it was demonstrated an increased in TNF levels in FM patients when compared to control group [8]. These data may indicate a possible specificity of location for the symptoms, and factors located in the skin perhaps are more important than in muscle, although more studies are needed to confirm such hypothesis.

Despite the contradictory results in the literature, the study of pro-inflammatory cytokines such as IL-6 and TNF in FM is relevant since it has been demonstrated a negative correlation between these cytokines and plasma levels of CoQ, a presumably deficient protein in FM [87-90]. Moreover, it has been demonstrated that oral supplementation of CoQ10 in FM patients decreased the TNF and ROS levels in addition of relieve symptoms [90]. The oral supplementation of CoQ10 in patients with breast cancer can also decreased the levels of inflammatory cytokines, such as IL-6 , IL-1 and IL-8 [91] and reduce the inflammatory process induced by ultraviolet radiation or IL-1 [92], while the CoQ10 deficient cause rise of TNF levels and hyperalgesia [90]. It is possible that the rise of some cytokines maybe secondary to the enhanced production of superoxide that happens in the deficient of CoQ [66,90].

As previously mentioned, a decrease in mitochondrial mass and the CoQ levels and an increase of ROS has been shown in BMCs of FM patients [87-90] and in turn ROS may trigger inflammation mediated by inflammasome [93], which it has been characterized as sensor of stress and metabolic damage. In an attempt to understand the involvement of mitochondrial dysfunction with inflammation, Cordero et al., conducted a study that showed mitochondrial dysfunction with reduced of protein mitochondrial complex, mitochondrial activity and CoQ. Additionally, they found an increase in gene expression NLRP3 and caspase-1, increased protein expression NLRP3 and cleavage of caspase-1, suggesting activation of inflammasome. In addition to observe increased IL-1β and IL-18 that may also activate the inflammasome. Furthermore, treatment with CoQ was able to reverse the inflammasome activation and levels of IL-1β and IL-18. These data, taken together, reinforce the role of oxidative stress in association with the inflammatory process that is often found in studies of FM patients.

Also regarding the involvement of oxidative stress in FM and inflammation it has been investigated the role of NO. This molecule has been related to pain and fatigue and there is also evidence to support its participation in mediating the inflammatory process [40,94-96]. In this context, NO is regarded as a potent anti-inflammatory agent, since it is able to suppress the activity of the nuclear transcription factor κβ (TNF κβ) one of the most important key factor in the installation of chronic inflammatory diseases once it's able to induce various cell types to release cytokines such as IL-1, IL-6, tumor necrosis factor α (TNF) as well as cell adhesion molecules and reactive oxygen species [40,94].

In this context some studies have used moderate aerobic exercise to increase the concentrations of NO to combat chronic inflammatory process in FM and chronic fatigue syndrome [96,97]. These exercises promote the generation of shear stress on the vascular wall, resulting in release of NO, particularly by eNOS enzyme [96]. This increase in NO concentration results in inhibition of the inflammatory TNF κβ decreasing mediators that are known to be associated with symptoms of pain and fatigue [94,98]. Thus, the prescription of aerobic exercise of moderate intensity appears to be an efficient alternative for the reduction of pain and fatigue experienced by FM patients [96,97]. However, a commitment to physical activity must be lasting, since one month after cessation of exercise, all symptoms returned to levels similar to those found before training [96].

Conclusion

The findings related to the role of NO in the pathophysiology of FM are still inconsistent and conflicting, although in most cases favoring the relationship. In the other hand, the findings related to the oxidative stress in FM are quite robust and homogeneous. Apparently, the reduced levels of NO and other enzymatic and non-enzymatic antioxidants evidenced in FM patients play an important role in the generation of oxidative stress, causing lipid peroxidation, mitochondrial damage and cell death. All these events together influence the manifestations and severity of the FM main symptoms, such as pain and fatigue. However, despite strong evidence that relates oxidative stress to the FM, it is still necessary to determine whether oxidative stress is the cause or effect of FM. Also it is important to consider the NO and oxidative stress at least as an important therapeutic targets.

More researches are needed to elucidate the mechanism of both oxidative stress and NO in the pathophysiology of FM syndrome, seeking primarily to clarify the cause/effect relationship.

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