Autohemotherapy with ozone as a possible effective treatment for Fibromyalgia

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ACTA REUMATOL PORT. 2019;44:244-249

ABSTRACT

Objective: The objective of this study is to evaluate the effectiveness of autohemotherapy with ozone in the management of fibromyalgia (FM).

Design: 20 FM patients (according to the criteria of the American College of Rheumatology), were treated with 10 sessions of ozone hemotherapy (2 sessions per week) with a concentration of 30-60 mcgr/ml. The health condition of the patients was evaluated before and after treatment, through the Fibromyalgia Impact Questionnaire (FIQ). Blood samples were obtained from all patients by venous puncture for biochemical routine analysis and serotonin levels in serum and the following peripheral blood mononuclear cells (BMCs) were isolated for oxidative stress quantification: reactive oxygen species (ROS) generation, and lipid peroxidation (LP) and protein carbonyl (PC) content, as these are signs of oxidative cell damage.

Results: All patients treated with ozone reported an improvement in sleep and mental alertness, a marked decrease of asthenia accompanied by a decrease of FIQ as well as tender points, and a moderate increase of serotonin levels. Also, an important decrease of LP and PC was observed; ROS also decreased, although less obvious, which indicates a reduction in oxidative stress levels.

Conclusions: The autohemotherapy with ozone in patients with FM showed an important decline of tender points and FIQ score, as well as a decrease of oxidative stress levels. This treatment allows patients to face life with greater vitality and less drug use, diminishing harmful side effects. Further investigation should be carried out, including groups with more patients and clinical trials, to elucidate the effect of ozone therapy in patients suffering from FM.

Keywords: Fibromyalgia; Ozone; Hemotherapy; Serotonin; Oxidative stress.

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INTRODUCTION

Fibromyalgia (FM) is a common chronic pain syndrome accompanied by other symptoms such as fatigue, headache, sleep disturbances, and depression. It is diagnosed according to the classification criteria established by the American College of Rheumatology (ACR),[1] revised in 2010[2]. Despite being a common disorder that affects at least 5 million individuals in the United States[3], its pathogenic mechanism remains unknown. Oxidative stress was proposed as a relevant event in the pathogenesis of this disorder[4,5]. Accordingly, several studies have reported high levels of lipoperoxides and protein carbonyl content[6,7], as well as decreased antioxidant capacity with low levels of superoxide dismutase (SOD), catalase (CAT), or glutathione peroxidase (GPx)[7-9]. In addition, previous research revealed a decrease in the amount of CoQ_{10}, a molecule involved in the mitochondrial respiratory chain and also known as a powerful membrane antioxidant in mononucleated cells from peripheral blood of FM patients[6,7,10].

Due to this etiopathological issue, combined with the fact that pharmacological treatment for FM produces mitochondrial damage[11], several non-pharmacological antioxidant therapeutic strategies have been tested, such as *Gingko biloba* supplementation[12], CoQ_{10} intake[13,14], moderate aerobic exercise[15], etc. Melatonin, a hormone with high antioxidant potential synthesized by pineal gland, was shown to reduce pain levels, de-

pression, and dream disorder[16, 17]. Moreover, it has been proved that vitamin C and D improve clinical symptoms in FM patients[18,19]. Thus, glutathione and N-acetylcysteine, both featuring antioxidant properties, have been used in these patients in addition to moderate exercise[20].

However, few treatments have been proved to be successful and approved by FDA (Food and Drugs Administration) or EMA (European Medicines Agency) for FM, such as duloxetine and pregabalin[21]. Besides these, exercise is still the main therapeutic strategy against this illness.

On the other hand, ozone (O_3) therapy, a procedure that helps the body activate its own immune system and free radical scavengers, is thought to act by exerting a mild, transient, and controlled oxidative stress when it reacts with lipids, promoting an up-regulation of the antioxidant system and a modulation of the immune system[22,23]. This moderate oxidative stress produced by O_3 induces the activation of the transcription factor Nrf2 (nuclear factor-erythroid 2-related factor 2), activating in turn the transcription of antioxidant response elements (ARE). This activation results in an increase of several antioxidant enzymes, including, but not limited to, SOD, GPx, glutathione S-transferase (GST), CAT, heme oxygenase-1 (HO-1), NADPH-quinone-oxidoreductase (NQO-1), and heat shock proteins (HSP). These enzymes act, directly or indirectly, as free radical scavengers, and have been proved to be important in the successful treatment of many diseases[23].

This procedure has been applied by Hidalgo-Tallón *et al.*[24] and Tirelli *et al.*[25], who observed beneficial effects of O_3 treatment in FM patients by rectal insufflations, including better FIQ, and a statistically significant improvement of pain and fatigue. In this sense, as far as we are concerned, only two papers have been published related to the use of O_3 by hemotherapy in FM, one of which featuring only 5 patients[24]. Tirelli *et al.* also treated patients with O_3 by autohemotransfusion, but despite the good results, the authors did not distinguish which of the two methods used (rectal insufflation and hemotherapy)[25] was in fact behind the success. Therefore, more investigation is needed to study to what extent O_3 is useful for improving symptoms in FM patients. The purpose of the present work is to investigate the protective effect of autohemotherapy on the health of 20 patients suffering from FM by analyzing oxidative stress parameters.

MATERIALS AND METHODS

PATIENTS

The study was performed with the informed consent of all participants and the approval of the local Ethical Committee. We studied 20 FM patients, all women, with a mean age of 47.5 (±11.0) years, mean disease duration of 13.6 years (±9.2), mean tender points of 14.9 (±3.1) and FIQ (Fibromyalgia Impact Questionnaire) of 59 (range 0-80). Patients were recruited from the *Unidad de Fibromialgia de la Policlínica Aljarafe* (Seville, Spain), where they were diagnosed by a specialist according to the ACR criteria (2010 revision[1, 2]). FM patients were then treated only with O_3 therapy in the Anti-aging Unit of *Clínica Infanta Luisa* (Seville) and nutritional counseling. Blood samples were collected before and after treatment for serotonin and oxidative stress parameter determinations, and for standard biochemical analyses.

The inclusion criteria for this study were the following: patients diagnosed of FM in the last 2–3 years, based on the current ACR diagnostic criteria[1,2], who did not consume any drug or vitamin/nutritional supplement during a 15-day period before the collection of the blood samples. Exclusion was subject to any of following circumstances: (i) acute infectious diseases in the previous 3 weeks, (ii) past or present neurological, psychiatric, metabolic, autoimmune, allergy-related, dermal or chronic inflammatory disease, (iii) undesired habits (e.g., smoking, alcohol, etc.), (iv) medical conditions that required glucocorticoid treatment, use of analgesics, antidepressant drugs, (v) past or current substance abuse or dependence, and (vi) pregnancy or current breast-feeding.

AUTOHEMOTHERAPY WITH O_3

This treatment is based on treating 150 ml of the patient's blood with 150 ml of O_3 followed by reinfusion in the donor, in a fast procedure. The O_3 generator used was Hyper Medozon Confort, Herrmann. Each patient received 10 sessions of autohemotherapy with O_3 twice a week. Concentration for the first three sessions was 30 µg/ml, 40 µg/ml for the fourth, 50 µg/ml for the fifth, and 60 µg/ml for the last five. Time of O_3 infusion in each session was 7-10 min. The protocol used was a modified version of ACEOOT-TRA-012B.

SEROTONIN DETERMINATION

Blood samples were extracted from patients by venous puncture one week before O_3 hemotherapy and one

week after treatment. The sera collected from FM patients were centrifuged at 3,000 r.p.m. for 15 minutes at room temperature. The super-natants were collected, and the samples were quickly frozen to -80ºC until analysis. Serotonin from those samples was measured by immunoassay using a commercial kit (Labor Diagnostica Nord; Nordhorn, Germany).

MEASUREMENT OF OXIDATIVE STRESS PARAMETERS

Peripheral blood mononuclear cells (BMCs) were purified from heparinized blood with isopycnic centrifugation by using Histopaque-1119 and Histopaque-1077 (Sigma Chemical Co., St. Louis, MO, USA).

Mitochondrial ROS generation in BMCs was assessed with MitoSOX™ (Invitrogen/Molecular Probes, Eugene, OR, USA) incubated with 1 mol/L MitoSox for 30 minutes at 37°C and washed twice with PBS. Cells were analyzed by flow cytometry (excitation at 510 nm and fluorescence detection at 580 nm).

Lipid peroxidation in BMCs was determined by analyzing the accumulation of lipoperoxides using a commercial kit from Cayman Chemical (Ann Arbor, Michigan, USA). TBARS are expressed in terms of malondialdehyde (MDA) levels.

Plasma protein carbonyl content was quantified by spectrophotometric measurement of 2, 4-dinitrophenylhydrazine derivatives of protein carbonyls. Samples were precipitated with trichloroacetic acid at a final concentration of 20%, centrifuged at 16,400 g for 10 minutes and protein pellets allowed to react with 2, 4-dinitrophenylhydrazine. Pellets were then dissolved in sodium hydroxide and the concentration of protein carbonyls measured spectrophotometrically at 360 nm, according to the method proposed by Harma et al.[27]. Results are expressed as nmol/ml.

STATISTICAL ANALYSIS

Sample size was calculated with respect to FIQ score. Z-score was 1.96 and was determined based on confidence level at 95% with margin of error of 5%, a power of 0.8, and an value of 0.05. The power analysis originally suggested that the sample size to be recruited should be of at least 38 subjects. However, after applying inclusion and exclusion criteria, the final number of patients was reduced to 20.

The mean ± standard deviation (SD) for continuous variables was executed by means of descriptive statistics. Paired T-Student test was performed to evaluate the changes after autohemotherapy with O_3. Descriptive statistics and tests were performed at a significance level of 0.05, and the power of the statistical test was 90% (P = 0.9), using the STATA software (version 12, 2011, StataCorp).

This study conforms to STROBE statement (Strengthening the Reporting of Observational Studies in Epidemiology)[28].

RESULTS

Before treatment, patients showed high levels of pain, asthenia and depression, as well as a high FIQ (54.6±11.3). After 8 weeks of treatment, patients presented an important decrease of tender points and FIQ (37.2±10.6) (Table I). All patients reported an improvement in sleep and mental alertness, a marked decrease of asthenia, and a reduction of episodes of alertness and episodes and intensity of headache.

No significant alteration of biochemical parameters in serum was detected after O_3 hemotherapy. The values for biochemical parameters measured before O3 hemotherapy were: glucose 93.2±11.9 mg/dL (normal values: 76–110), urea 32.1±8.7 mg/dL (n.v.: 10–45), uric acid 3.2±0.4 mg/dL (n.v.: 2.5–7.5), total protein 8.4±2.3 g/dL (n.v.: 6.6–8.7), creatinine 0.7±0.2 mg/dL (n.v.: 0.5–1.1), aspartate aminotransferase 27.4±6.2 mU/mL (n.v.: 10–40), alanine aminotransferase 32.4±9.7 mU/mL (n.v.: 10–40), gammaglutamyl transferase 39.0±12.4 mU/mL (n.v.: 11–49), alkaline phosphatase 148±19.4 mU/mL (n.v.: 90–258), total cholesterol 220.0±10.6 mg/dL (n.v.: <220), HDL 57.0±6.0 mg/dL (n.v.: >35), LDL 135±10.2 mg/dL (n.v.: <150) and triglycerides 161±19.5 mg/dL (n.v.: 150–200). The values after treatment were: glucose 90.1±12.2 mg/dL (normal values: 76–110), urea 31.2±9.1 mg/dL (n.v.: 10–45), uric acid 3.1±0.5 mg/dL (n.v.: 2.5–7.5), total protein 8.3±2.5 g/dL (n.v.: 6.6–8.7), creatinine 0.7±0.2 mg/dL (n.v.: 0.5–1.1), aspartate aminotransferase 28.5±6.1 mU/mL (n.v.: 10–40), alanine aminotransferase 31.3±10.3 mU/mL (n.v.: 10–40), gammaglutamyl transferase 38.0±11.1 mU/mL (n.v.: 11–49), alkaline phosphatase 152±21.4 mU/mL (n.v.: 90–258), total cholesterol 201±11.12 mg/dL (n.v.: <220), HDL 59.0±6.7 mg/dL (n.v.: >35), LDL 128±9.1 mg/dL (n.v.: <150) and triglycerides 159±21.5 mg/dL (n.v.: 150–200). On the other hand, patients showed higher serotonin values in serum after treatment (mean: 60 ng/ml [±0.4]) in comparison to those found before treatment (mean: 53 ng/ml [±0.2]).

In relation to oxidative stress, after 8 weeks of treatment with O_3, an important decrease of malondialdehyde and protein carbonyl was observed, as well as a moderated reduction of ROS, which indicate a global decrease of the oxidative stress with respect to the level that had been observed in these patients before treatment (Table I).

No side effects were detected in patients after O_3 therapy treatment.

DISCUSSION

One of the main problems of FM is the lack of useful treatments, so specialists are forced to offer drugs as symptomatic treatment, leading sometimes to the aggravation of illness provoked by side effects. Many of these drugs induce mitochondrial damage and produce oxidative stress[11], and are not appropriate for patients with signs of mitochondrial dysfunction or elevated oxidative stress. FM patients are especially sensitive to oxidative damage since they usually show high levels of oxidative stress and mitochondrial damage[7, 10]. Among these drugs, tricyclic anti-depressant stands out, like amitriptyline, a common drug for analgesic treatment in FM patients. We have previously described that amitriptyline induces mitochondrial dysfunction, reduction of CoQ_{10} level, and high oxidative stress; co-treatment with antioxidants restored mitochondrial damage provoked by this anti-depressant[29].

In relation to this issue, it has been demonstrated that antioxidant treatments (e.g. vitamin E, SOD and CoQ_{10}) are beneficial as a therapy for mitochondrial illnesses[5]. Interestingly, one of the most accepted treatments for FM patients is moderate physical exercise[15]. This is based on the fact that aerobic exercise increases mitochondrial biogenesis and mitochondrial size, and for this reason it is proposed as alternative therapeutic strategy in diseases with mitochondrial dysfunction[30]. In this work, we have described the antioxidant effects of O_3 hemotherapy, with a reduction in ROS generation, and a subsequent decrease in oxidative damage in lipids and proteins, improving redox homeostasis. These results may contribute to the improvement in the symptoms of FM described in the patients under study.

However, serotonin is an important modulator of pain perception, sleep and mood. In a previous paper, our group has correlated the decrease of pain threshold and the presence of general pain, common in FM patients, with a decrease in serotonin values in serum. This suggests that the measurement of serotonin level is a useful indicator to evaluate the severity of FM symptoms[31]. In the present work, a moderate increase in serotonin levels have been detected in patients treated with O_3, which may have also contributed to improve symptoms of this disease, expressed by a significant decrease in tender points and FIQ score. This could be explained in part due to balanced diet tips given to patients before the treatment with O_3. It has been reported that diet guidelines containing tryptophan and antioxidant components may have a special relevance by affecting inflammatory signaling cascades, including tryptophan breakdown, and could increase

TABLE I. CLINICAL AND BIOCHEMICAL DATA IN FM PATIENTS PRE AND POST TREATMENT WITH OZONE THERAPY

Items	Baseline (±SD)	Endpoint (±SD)
Age (years old)	47.5 (±11.0)	–
Tender points	14.9 (±3.1)	7.0 (±2.1)*
Duration of the disease (years)	13.6 (±9.2)	–
BMI (Kg/m^2)	27.98	–
FIQ total score (range 0-80)	54.6 (±11.3)	37.2 (±10.6)*
Serotonin in serum (ng/ml)	53.0 (±0.2)	60.0 (±0.4)*
MDA (nmol/million cells)	26.4 (±3.7)	11.1 (±2.4)*
Protein carbonyl (nmol/L)	40.8 (±7.6)	26.2 (±5.4)*
ROS (a.u.)	10.6 (±1.6)	7.6 (±2.5)*

BMI: body mass index; FIQ: fibromyalgia impact questionnaire; MDA: malondialdehyde; ROS: reactive oxygen species. a.u: Arbitrary units. All values are mean (±SD). Serotonin reference in female: 70-270 ng/ml±0,1. MDA reference for lipid peroxidation: 6±1. Protein carbonyl reference: 18.3 ±2.2. ROS levels reference: 5.8 au± 0.4).

blood and brain tryptophan availability for serotonin production[32].

In a previous report, Hidalgo-Tallón *et al.*[33] carried out an open-label pilot study with O_3 therapy by rectal insufflation for the treatment of FM, observing a significant improvement in symptoms of FM patients after four weeks of treatment, with few side effects. Tirelli *et al.* also used rectal insufflation in 10 out of 65 patients treated in their study, the remaining 55 patients were treated with autohemotransfusion[25]. The hemotherapy with O_3 has been used for others illnesses because of its anti-inflammatory and antioxidant effects, which contribute to the regulation of endogenous nitric oxide concentrations, the maintenance of an adequate cellular redox balance, and the improvement in oxygen diffusion[34-37]. Nevertheless, almost none of the papers published are related to O_3 therapy administered intravenously in FM patients. In this sense, Tirelli *et al.* treated some patients with O_3 by rectal insufflation and some others by autohemotherapy. Although they found a significative improvement of symptoms (pain and fatigue) in 45 patients (70%), they did not make a distinction in the therapy method that was used when analyzing the results[25]. Moreover, Borrelli and Bocci have reported the effect of autohemotherapy with O_3, but only in 4 patients[26]. They administrated natural antioxidant to patients, which could affect the results obtained, as can be deduced from the investigation of Inal *et al.*, when they employed a combined management of O_3 plus CoQ_{10}[38]. Nevertheless, future studies should be performed in order to identify the relationship between extra antioxidants provided in the diet and the benefit in FM patients treated with O_3. In this work, we have studied 20 patients receiving O_3 through the same therapy, and without supplementary treatments.

In conclusion, the autohemotherapy with O_3 applied to FM patients has resulted in a significant decrease of tender points and total FIQ score. In addition, we observed a decrease in oxidative stress, which affords this treatment enough relevance to be considered by physicians when treating FM patients. Nevertheless, further investigation should be performed in the context of a clinical trial, and with a larger set of patients.

ACKNOWLEDGMENTS

This work has been partially supported by the Spanish research association FOICAM. We thank all FM patients for their essential role in this investigation.

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