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Medical ozone increases methotrexate clinical response and improves cellular redox balance in patients with rheumatoid arthritis

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ABSTRACT

Medical ozone reduced inflammation, IL-1β, TNF-α mRNA levels and oxidative stress in PG/PS-induced arthritis in rats. The aim of this study was to investigate the medical ozone effects in patients with rheumatoid arthritis treated with methotrexate and methotrexate+ozone, and to compare between them. A randomized clinical study with 60 patients was performed, who were divided into two groups: one (n=30) treated with methotrexate (MTX), folic acid and Ibuprophen (MTX group) and the second group (n=30) received the same as the MTX group+medical ozone by rectal insufflation of the gas (MTX+ozone group). The clinical response of the patients was evaluated by comparing Disease Activity Score 28 (DAS_{28}), Health Assessment Questionnaire Disability Index (HAQ-DI), Anti-Cyclic Citrullinated (Anti-CCP) levels, reactants of acute phase and biochemical markers of oxidative stress before and after 20 days of treatment. MTX+ozone reduced the activity of the disease while MTX merely showed a tendency to decrease the variables. Reactants of acute phase displayed a similar picture. MTX+ozone reduced Anti-CCP levels as well as increased antioxidant system, and decreased oxidative damage whereas MTX did not change. Glutathione correlated with all clinical variables just after MTX+ozone.

MTX+ozone increased the MTX clinical response in patients with rheumatoid arthritis. No side effects were observed. These results suggest that ozone can increase the efficacy of MTX probably because both share common therapeutic targets. Medical ozone treatment is capable of being a complementary therapy in the treatment of rheumatoid arthritis.

1. Introduction

Rheumatoid arthritis (RA) is the commonest inflammatory joint disease, afflicting about 1% of the world population.

The ultimate therapeutic goal in RA treatment is remission or at least low disease activity, which may not always be achieved with methotrexate monotherapy, so that combination therapy appears to be better. Recent reports found that most dissatisfied arthritis patients are likely to seek the option of complementary and alternative medicine (Efthimiou et al., 2010).

On the other hand, the combination of carnosine (antioxidant) with methotrexate decreased inflammation in rheumatoid arthritis model in rats more effectively than treatment with methotrexate alone. This result is in compliance with other studies on combination of naturally occurring substances and methotrexate, where the combination proved more effective than monotherapy (Bauerova et al., 2010). A combination of MTX with drugs that decrease inflammation in RA and also show antioxidant properties would be better than MTX monotherapy.

MTX remains the first line drug for the treatment of RA. Although some mechanisms have been proposed, most of the available evidence supports the adenosine theory which plays a role in MTX efficacy in RA (Roopjet et al., 2014). Adenosine interacts with specific cell surface receptors, with subsequent inhibition of inflammation mediators (Cronstein, 2005).

The Reactive Oxygen Species (ROS) are another factor involved in RA. A great amount of ROS produced in arthritic joints (Lisa et al., 2012) has contributed to the fact that many studies associate oxidative stress with RA. Indeed, oxidative stress is closely related

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with RA and the maintenance of a proper redox balance seems to be a critical step in order to improve the clinical outcome.

At present, there is no available direct cure for RA; the main goals of the treatment are therefore to ameliorate the symptoms of the disease (i.e., diminish pain and decrease inflammation and joint destruction). In addition, the majority of these drugs have numerous side effects (Kyra et al., 2007).

Medical ozone is an ozone/oxygen mixture administered at low concentrations. It is able to reestablish cellular redox balance and increase adenosine availability through an ozone oxidative pre-/postconditioning mechanism (León et al., 1998). The proposed mechanism has been validated in pathological conditions such as ischemic syndrome, diabetes and diabetic foot, disc hernia, pain and other diseases (León, 2014).

Adenosine and redox status are common therapeutic targets of MTX and medical ozone so it is possible to hope that the combination of both therapeutic concepts could increase the MTX clinical response. Previous to this work ozone protective effects on PG/PS- induced arthritis in rats on inflammation and reduction of IL-1β and TNF-α mRNA levels were demonstrated (Jaqueline et al., 2013).

Taking into account that medical ozone shares therapeutic targets with MTX, the aim of this project was to study the effects of medical ozone in patients with RA being treated with methotrexate and methotrexate+ozone (rectal insufflations), and to compare both groups with regard to MTX clinical response as well as to investigate whether medical ozone antioxidant properties are associated with the clinical outcome.

2. Materials and methods

2.1. Study design

This randomized controlled clinical study was approved by the joint institutional review board (Scientific and Ethics Committees from the National Institute of Rheumatology, Ministry of Public Health, Cuba, and Pharmacy and Food Institute, University of Havana, Cuba) in accordance with the principles of the Declaration of Helsinki (2005). All patients gave their informed consent to enrollment after receiving adequate information concerning the study (characteristics of the study, benefits and possible side effects). Before enrollment, all participants attended a training program to familiarize them with the study objectives and treatment plans. The personnel involved emphasized that all participating physicians would treat each patient according to the randomized scheme of treatment through a Research Randomizer Form.

To calculate the size of the sample, the Medstat Systems, Inc. (version 2.1, 1989; Fridley, MN, USA) method was used. The statistical difference between the beginning and the end of ozone therapy was 0.2 with a type 1 error of 0.05 (Levy and Lemeshow, 1991). The target level of enrollment was determined at 27 patients. Assuming that 10% of the patients studied would be lost to follow-up, 30 patients were included.

Inclusion criteria: Adult patients (>18 years) of both sexes and different ethnic origins with a diagnosis of RA who fulfilled the revised American Rheumatism Association's (Arnett et al., 1988) criteria for RA (morning stiffness, swelling of hand joints, swelling of three or more joints, symmetric swelling of joints) were eligible to participate in the study. Patients of the National Institute of Rheumatology, Ministry of Public Health, Cuba who accomplished the following criteria were chosen: Disease Activity Score 28 (DAS $_{28}>3.2$ y ≤ 5.1) whose examination was carried out under blinded conditions by a physician different to the one who selected the patients according to a randomized scheme of treatment and a preliminary brief medical history. The Health Assessment Questionnaire-Disability Index: (HAQ-DI, according to the validated Spanish version) (Cardiel et al., 1993), "C" Reactive Protein (CRP >6 mg/l in serum), Erythrocyte Sedimentation Rate (ESR >8 mm for males and 16 nm for females) and anti-Cyclic Citrullinate Peptides (anti-CCP >10 U/ml in serum) as well as patients with disease duration longer than five year were included. The exclusion criteria were: patients with any history of chronic conditions such as liver disease, diabetes mellitus, respiratory disorders, cardiovascular diseases and alcohol usage and smoking were not included in the study. Patients with overlapping syndrome, cancer, or other associated autoimmune disorders or who were pregnant were also excluded. Those patients who had been receiving corticosteroid agents and were under treatment with disease modifying anti-rheumatic drugs and anti-TNF or other biological agents for at least 3 months before the study date were also excluded.

The patients were randomized into two different groups of treatment: (MTX group), MTX 12.5 mg, intramuscular (i.m.), once/week (every Monday from 9:00–10:00 in the morning)+Ibuprophen (400 mg, oral), one Tablet each 8 h+Folic Acid (5 mg, oral), one Tablet/day from Wednesday to Saturday. (MTX+ozone group), same MTX group+medical ozone which was generated by an OZOMED unit, Cuba. 20 treatments by rectal insufflations (five/week from Monday to Friday). 25 mg/l to 40 mg/l of ozone in stepped application and in increasing order were administered as follows:

1st week: 25 mg/l, 100 ml; 2nd week: 30 mg/l, 150 ml; 3rd week: 35 mg/l, 200 ml; 4th week: 40 mg/l, 200 ml.

Medical personnel were instructed to report all adverse reactions, whether described in the package circulars of the study medications or not.

2.1.1. Evaluation of disease activity

Changes in the evolution of disease, that is, clinical improvements through suitable indices of activity (clinical parameters) as well as anti-CCP antibodies and redox status determinations before the beginning and at the end of clinical study (21 days) were assessed. Each patient was his/her own control (i.e. before medical ozone treatment).

The main variables considered were:

Clinical parameters: DAS$_{28}\leq 3.2$ (Prevoo et al., 1995), decrease of HAQ-DI and a reduction of pain intensity (VAS $\geq 50\%$). A visual analogical scale (VAS) from '10' to '100' was evaluated. This was classified as '10' (minimum pain intensity) and '100' (maximum pain intensity). No pain was considered as "0" as well as a decrease in the reactants of acute phase and anti-CCP.

Secondary variables considered were: (a) Serum levels of injury markers such as advanced oxidation protein products (AOPP), nitric oxide (NO), total hydroperoxides (TH) and malondialdehyde (MDA). (b) Serum levels of protective redox markers such as reduced glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) activities. c) Side effects.

A result was considered good when MTX+ozone group decreased ($P<0.05$) with regard to MTX group at the end of the study (21 days) in: DAS$_{28}$, HAQ-DI, pain intensity, acute phase reactants and auto antibody anti-CCP levels. An increase in endogenous antioxidants (GSH, SOD and CAT) and a decrease in injury redox markers (NO, AOPP, TH and MDA) were also considered to be good results.

The therapeutic response was considered successful if 70% of the patients treated with MTX+ozone had a positive outcome, taking into account the main variables, and if this improvement was 30% higher than that in the patients treated with MTX by itself.