Clinical evidence of ozone interaction with pain mediators

Lamberto Re, MD, PhD, Gregorio M. Sánchez, Pharm. D, PhD, Nabil Mawsouf, MD, PhD.

ABSTRACT

الأهداف: دراسة الأدلة السريرية التي يمكن أن تؤكد تأثير حقن الأوزون تحت الجلد في تخفيف الشعور بالألم الشديد وذلك بسبب قدرته على تعديل أهداف معينة من الألم.

الطريقة: أُجريت هذه الدراسة في عيادة ميدينات، كاميرانو، أنكونا، إيطاليا وذلك خلال الفترة من أكتوبر 2004م إلى أكتوبر 2009م، وشملت 52 مريضاً مؤهلاً للمشاركة في هذه الدراسة. لقد تم تحليل الصور البانورامية التي أُخذت للمناطق المؤلمة وغير المؤلمة والتي تم علاجها بحقن خليط من الأوزون والأكسجين بهدف تحديد شدة الاحمرار.

النتائج: أظهرت النتائج أن العلاج بحقن الأوزون قد قام بزيادة درجة الاحمرار في المناطق المؤلمة بنسبة (83±5%) (p<0.05) وذلك بالمقارنة مع المناطق الغير مؤلمة بالجهة المقابلة من الجسم (7±6%).

خاتمة: أشارت الدراسة إلى أن درجة احمرار المناطق التي تم حقنها بالأوزون يمكن إرجاؤها ولو جزئياً إلى تفاعل الأوزون مع وسطاء الألم، حيث تتضمن هذه العملية كلاً من الوسطاء المسببة للألم أو مستقبلات الألم. تشتمل آلية تخفيف الألم بواسطة خليط الأوزون والأكسجين على خطوتين مستقلتين وهما: آلية قصيرة الأجل والتي تكمن في الأكسدة المباشرة للجزئيات الحيوية، وآلية طويلة الأجل وتكمن في تنشيط عمل مضادات الأكسدة. ونحن بحاجة إلى المزيد من الدراسات لإثبات آلية عمل الأوزون وفعاليته في تخفيف الألم من الناحية الحيوكيميائية.

Objectives: To address the clinical evidence that may support the fact that subcutaneous administration of ozone (O_3) has anti-hyperalgesic effects probably acting by modifying specific pain targets.

Methods: Fifty-two patients attending the Medinat Clinic, Camerano, Ancona, Italy between October 2004 to October 2009 were eligible to participate in the study. Panoramic photos of painful and not painful areas submitted to oxygen (O_2)/O_3 treatment injection were analyzed to detect the intensity of erythema.

Results: The erythematic areas after O_3 subcutaneous puncture showed significant (p<0.05) increment (83±5%) in the painful area versus 7±6% in the contralateral area.

Conclusion: The surrounding erythema observed during O_3 intervention should explain at least in part, its interaction with some pain mediators. This may involve algesic mediators or receptors. The analgesic mechanism induced by O_2/O_3 may involve 2 independent steps: a short-term mechanism that may correspond with the direct oxidation on biomolecules, and a long-term mechanism that may involve the activation of antioxidant pathways. Further studies are needed to support the biochemical analgesic mechanism of O_3 therapy.

Saudi Med J 2010; Vol. 31 (12): 1363-1367

From the Ozone Therapy Unit (Re, Sanchez), Medinat Clinic and Department of Pharmacology (Re), University of Ancona, Ancona, Italy, and Ozone Therapy Unit (Mawsouf), National Cancer Institute, Cairo University, Cairo, Egypt.

Received 4th October 2010. Accepted 8th November 2010.

Address correspondence and reprint request to: Prof. Lamberto Re, Ozone Therapy Unit, Medinat Clinic, Via Fazioli 22, Camerano 60021, Ancona, Italy. Tel. +39 (71) 731076. Fax. +39 (71) 731347. E-mail: lambertore@univpm.it

Since the studies by Wentworth et al in 2002,[1] triatomic oxygen or ozone (O_3) was assigned to the biological group of reactive oxygen species, and apart from hydrogen peroxide (H_2O_2), superoxide radicals and their sequel products were seen as part of the oxidative defense against infection. It is apparently also released as a response to inflammations.[1] Attention has been focused on the use of medical O_3, and on its rationale in pain management in the last few years.[2-5] There are main evidence on the regulative role of O_3 in the antioxidant enzymes, nitric oxide pathways, and other subcellular activities that could be modulated by low doses.[6-8] The pain relief effect of O_3 was shown in patients affected by low back pain in lumbar and cervical disk herniation without any significant complications when topically applied.[2,5,9] Subjective symptoms

including pain is probably one of the most important clinical sign as it is often this complaint that motivates patients to seek treatment. There are many variations in human responses to O_3 exposure that remains poorly understood. Well-documented responses to short-term high doses of O_3 exposures (by air ways) include: deficits in pulmonary function, increased respiratory symptoms, increased hospital admissions for respiratory diseases, such as asthma, influenza, pneumonia, and chronic obstructive pulmonary disease.[10] There are also increased rates of daily mortality,[11] and promotes increased vascular dysfunction, oxidative stress, mitochondrial damage, and atherogenesis.[12] However, a small O_3 dose well-calibrated against the potent antioxidant capacity of blood can trigger several useful biochemical mechanisms, and reactivate the antioxidant system.[13] Apart from the well-documented oxygen release effect and the immunomodulatory effect of O_3, up-regulation of the antioxidants with their cell-protective effect is turning out to be the most important active mechanism in O_3 therapy.[14] The O_3 treatment shows long-term anti-inflammatory effects with apparent low toxicity.[15] Ozone therapy avoids damage caused by hepatotoxic or nephrotoxic agents, and increases the endogenous antioxidant system activities in endotoxic and septic shock models.[14,16] However, there was only one study aimed at identifying the possible molecular mechanisms of the anti-allodynic/hyperalgesic effect of O_3 and the possible involvement of some inflammatory and apoptotic pathways.[17] The aim of this study was to provide preliminary clinical evidence that may support the fact that a single subcutaneous administration of O_3 has anti-hyperalgesic effects probably acting by modifying specific pain targets.

Methods. This is a prospective clinical trial approved by the Scientific and Ethics Committee of the Institution in accordance with the principle of the Declaration of Helsinki.[18] All patients signed an informed consent form before being enrolled. All patients were given adequate information (characteristics of the study, benefits and possible side effects). Before enrolling, all participants attended a training program to familiarize with the study objectives and treatment plans. Fifty-two adult patients of both genders, all Caucasian, with a diagnosis of shoulder, cervical, lumbar spine, or low back pain attending the Medinat Clinic, Ancona, Italy between October 2004 and October 2009 were eligible to participate in the study. Baseline data was obtained from its clinical record, and after an individual interview. The following exclusion criteria were adopted: severe septic conditions, hypersensitivity to the medication to be used, hepatic dysfunction, renal failure (serum creatinine level >1.32 µmol/L), pregnancy, cancer or other serious disease, inability to cooperate with the requirements of the study, recent history of alcohol or drug abuse, current therapy with any immunosuppressive agent or anticonvulsant, concurrent participation in another clinical study or current treatment with an investigational drug, and computed tomography or magnetic resonance evidence of a herniated disc fragment with symptoms of motor and/or sphincter disturbance, evidence of disc herniation corresponding to clinically severe motor deficit and/or sphincter disturbance.

Patients were treated with an oxygen/O_3 (O_2/O_3) mixture (generated just before administration by Ozonozan Alpha Plus Device, Iffezheim, Germany) with an O_3 mixture of 25-50 mL (O_3 concentration of 8-12 µg/L, and 3-5 mL per application). The O_3 obtained from medical grade oxygen represents approximately 0.4-0.5% of the gas mixture. The O_3 concentration was measured using a built-in ultraviolet spectrophotometer at 254 nm. Percutaneous injection was carried out using 30 mL disposable syringes (O_3 resistant), and 30 G x 1/2 disposable needle inserted in the surrounding affected area (local puncture). The O_3 inhalation was avoided during the treatment and O_3 was injected in the painful area (according to the patient's reference), and in the contralateral corresponding areas. One minute before injection, panoramic photography of the area was carried out. Following image acquisition, the images were analyzed blindly using a commercial image processing software program (Photoshop version 7.0, Adobe Systems, Mountain View, CA, USA). Measurements were made by manually outlining the margins of erythema areas in triplicate. Contralateral area was defined as an equivalent area to the therapeutic area in the opposite side. Erythema areas were expressed as a percentage of the total measured area.

The outliers preliminary test for detection of error values was initially applied. Afterward, data were analyzed for normality using Shapiro-Wilks W test followed by Levene test of homogeneity of variance. In addition, descriptive statistics was carried out. Data were presented as mean ± standard deviation. Values were analyzed by Kruskal-Wallis non-parametric ANOVA followed by the Mann-Whitney test for analysis of individual differences. Differences were considered significant when $p<0.05$. The Statistical Package for Social Sciences version 11 (SPSS Inc, Chicago, IL, USA) software was used for all statistical analyses.

Disclosure. This study was partially funded by the Department of Pharmacology, University of Ancona, Ancona, Italy.

Results. Baseline characteristics of patients as shown in **Table 1** showed no differences in gender, and a median of 56 years (14-97 years). Analgesic and anti-inflammatory therapy was concomitant in 61.5% of patients, physical therapy in 27%, and a minor incidence was detected in the use of vitamins or supplements (2%). Cardiovascular diseases (29%), diabetes (27%), and hypertension (25%) was the main background pathological diseases, and in addition, 2% of patients was submitted to previous chirurgical intervention (connected with the painful areas). Patients suffering from chronic pain (87%) were prevalent compared to those suffering from neuropathic pain (13.4%). Lumbar spine (44.2%), low back (25%) and cervical (21.1%) was the prevalent localization of the pain. All patients enrolled belong to the Caucasian ethnic group. The analysis of the erythematic areas after O_3 puncture showed a significant increment ($p<0.05$, 83 ± 5%) of erythema in the painful area versus 7 ± 6% in the contralateral area. The intensity of erythema remains 16 ± 7 minutes in both painful and contralateral control area. A representative photo of one patients (**Figure 1**) show this trend. No differences was found between gender or age in the intensity of the erythematic areas.

Discussion. Tissue damage caused by injury, disease, or inflammation results in the release of endogenous algogenic or algesic chemicals, and these may reach the extracellular fluid. Most mediators surrounding nociceptors in painful condition have a role in vasodilatation-vasoconstriction mechanism leading to hyperalgesia. Among these mediators are mast cell amines, kinins, substance P and neurokinins, prostaglandins (PG) E2 and I2, nitric oxide, platelet-activating factor adenosine and cytokines, all of which are released during inflammatory responses accompanying tissue damage.[19] In addition, a large number of inflammatory and signaling substances, such as tumor necrosis factor (NF) and interleukins (ILs[-1beta, IL-6, and IL-8),[20] may also play a role in the development of sciatica, hernia-derived pain, and back pain. Myelinated A delta fibers and unmyelinated C fibers to the dorsal root ganglion, independent of nociceptor stimulus conducts pain impulses. This impulse continue by way of the spinothalamic tract to the thalamus and the somatosensory cortex. The disc in response to stimulation of the nociceptors may increase its sensitivity, resulting in a non-functional response, such as peripheral sensitization.[21] When a disc degeneration leads to disc herniation, the adjacent nervous system structures, such as the nerve roots, or the dorsal root ganglion can be affected causing neuropathic pain of mechanical or biochemical origin. During the disc deterioration other spinal structures are damaged, such as facet joints, ligaments and muscles, which can also become pain generators. Thus, disc degeneration may be responsible for the development of chronic low back pain without being the actual pain focus.[22]

Peripheral sensitization should be avoided by O_3 or O_3 mediators. Recent studies have provided strong evidence

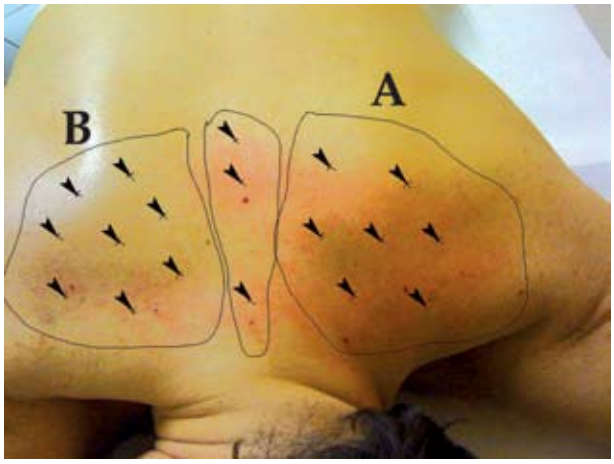

Figure 1 - Representative photograph of a 36 year-old female patient suffering from dorsal scapular chronic pain after one minute of puncture with ozone 8 µg/mL, 2-3 mL per puncture zone (arrowheads). (**A**) Right scapular area (83 ± 5% of erythema) was the painful area referred by patient. (**B**) Left scapular area (7 ± 6% of erythema) was the contralateral control area.

Table 1 - Baseline patient characteristics.

Characteristics	Variables	Total (n=52) n (%)
Age (years)	Median	56
	Minimum	14
	Maximum	97
Gender	Female	24 (46.1)
	Male	28 (54.0)
Concomitant treatment	Analgesic/anti-inflammatory	32 (61.5)
	Vitamins/supplements	1 (2.0)
	Physical therapy	14 (27.0)
Clinical records	Surgery*	1 (2.0)
	Hypertension†	13 (25.0)
	Renal dysfunction‡	4 (8.0)
	Diabetes§	14 (27.0)
	Cardiovascular disease¶	15 (29.0)
Pain localization	Shoulder	5 (10.0)
	Cervical	11 (21.1)
	Lumbar spine	23 (44.2)
	Low back	13 (25.0)
Pain classification	Chronic pain	45 (87.0)
	Neuropathic pain	7 (13.4)

*previous surgery intervention related to the affected side, †hypertension was defined as elevation of systolic (>140 mm Hg), and/or diastolic (>90 mm Hg) blood pressure, ‡renal dysfunction - increase in serum creatinine >133 µM, §fasting overnight plasma glucose concentration >3.58-5.6 mM, ¶cardiovascular disease was diagnosed by thorough history and physical examination

that ozonized low density lipoprotein inhibited NF-kappaB and IL-1 receptor-associated kinase 1 (IRAK-1) signaling, which may impair immune function.[23] A similar mechanism should function in O_3 therapy. The oxidation of IL, IL receptors or nuclear factors should block cyclooxygenase (COX) 2 expression, and as a consequence the biochemical pathway of pain. In fact, in clinical experience, radiological evidence may or not be relevant in patients with pain derived from radiculopathy. However, 80% of success is achieved in pain derived from radiculopathy.[5] Furthermore, recent scientific data demonstrated the physiological presence of O_3-like mediators during inflammation, indicating O_3 as a new biomolecule endowed with striking effects, which must be considered and studied following new strategies with newly constructed randomized-standardized clinical studies.[24] The target of the effects of non-steroidal anti-inflammatory drug (NSAIDs) was to controll the neural cell production of COX-1 and COX-2 derived PG (F2α), PG E2 acting on EP1, EP3, EP4 and IP-prostaglandin receptors in afferent pathways and of nitric oxide, although indirect effects occur from these drugs on the production of mediators that underlie the inflammatory reactions in the periphery, resulting in nerve sensitization and initiation of pain responses via afferent stimulation.[19] In chronic pain management, the adverse effects of NSAIDs become the main drawback in contrast with the low incidence of side effects reported for O_3 therapy.

The surrounding erythema observed during O_3 intervention (**Figure 1**) should explain at least in part its interaction with some pain mediators. This may involve algesic mediators or receptors. It is interesting to highlight that in patients with low back pain after O_2/O_3 puncture were possible to see the dermatome designed in red (erythema). In this case, O_2/O_3 acts as a kind of "pain developer". Most of the mediator surrounding nociceptors in painful condition has a role in vasodilatation-vasoconstriction mechanism, and probably O_3 disrupt this mechanism to induce a superficial local vasodilator effect observed as erythema. After the O_3 injection (16 ± 7 minutes) the erythematic area became normal, and pain relief sensation was referred by patients. This fact can be explained, at least in part, by the interaction of O_3 with pain mediators. Of paramount interest was the fact that during O_3 therapy if only the painful area was treated, after one-2 weeks patients experience almost total pain relief, however the contralateral area (previously normal) became painful. Simultaneous treatment of painful area and contralateral area avoids this "pain migratory effect".

We hypothesized that measuring blood flow by Doppler flow techniques, changes in dermal temperature, or the measurement of mediator concentrations after O_3 treatment will contribute to clarify the exact mechanism originating temporary erythema in painful areas.

This study is only limited to the observation of the phenomenon, however a deeper research is needed to clarify the biochemical pathways involved. The analgesic mechanism O_2/O_3 may involve 2 independent steps: a short-term mechanism that may correspond with the direct oxidation on biomolecules, and the long-term mechanism that may involve the activation of antioxidant pathways. The analgesic effect of O_3 in right doses is a clinical fact, however further studies are needed to support the biochemical analgesic mechanism of O_3 therapy including the assays of the variations of pain mediators in painful areas.

References

1. Wentworth P Jr, Wentworth AD, Zhu X, Wilson IA, Janda KD, Eschenmoser A, et al. Evidence for the production of trioxygen species during antibody-catalyzed chemical modification of antigens. ***Proc Natl Acad Sci U S A*** 2003; 100: 1490-1493.
2. Paoloni M, Di Sante L, Cacchio A, Apuzzo D, Marotta S, Razzano M, et al. Intramuscular oxygen-ozone therapy in the treatment of acute back pain with lumbar disc herniation: a multicenter, randomized, double-blind, clinical trial of active and simulated lumbar paravertebral injection. ***Spine*** 2009; 34: 1337-1344.
3. Bo W, Longyi C, Jian T, Guangfu H, Hailong F, Weidong L, et al. A pyogenic discitis at c3-c4 with associated ventral epidural abscess involving c1-c4 after intradiscal oxygen-ozone chemonucleolysis: a case report. ***Spine (Phila Pa 1976)*** 2009; 34: E298-E304.
4. Dahnhardt JE, Gygax M, Martignoni B, Suter P, Lussi A. Treating sensitive cervical areas with ozone. A prospective controlled clinical trial. ***Am J Dent*** 2008; 21: 74-76.
5. Re L, Martínez-Sánchez G, Malcangi G, Mercanti A, Labate V. Ozone Therapy: a clinical study on Pain Management. ***International Journal of Ozone Therapy*** 2008; 7: 37-44.
6. Ajamieh HH, Menendez S, Martinez-Sanchez G, Candelario-Jalil E, Re L, Giuliani A, et al. Effects of ozone oxidative preconditioning on nitric oxide generation and cellular redox balance in a rat model of hepatic ischaemia-reperfusion. ***Liver Int*** 2004; 24: 55-62.
7. Martinez-Sanchez G, Al-Dalain SM, Menendez S, Re L, Giuliani A, Candelario-Jalil E, et al. Therapeutic efficacy of ozone in patients with diabetic foot. ***Eur J Pharmacol*** 2005; 523: 151-161.
8. Bocci V, Borrelli E, Travagli V, Zanardi I. The ozone paradox: ozone is a strong oxidant as well as a medical drug. ***Med Res Rev*** 2009; 29: 646-682.
9. Gallucci M, Limbucci N, Zugaro L, Barile A, Stavroulis E, Ricci A, et al. Sciatica: treatment with intradiscal and intraforaminal injections of steroid and oxygen-ozone versus steroid only. ***Radiology*** 2007; 242: 907-913.
10. Yamazaki S, Shima M, Ando M, Nitta H. Modifying effect of age on the association between ambient ozone and nighttime primary care visits due to asthma attack. ***J Epidemiol*** 2009; 19: 143-151.
11. Thurston GD, Ito K. Epidemiological studies of acute ozone exposures and mortality. ***J Expo Anal Environ Epidemiol*** 2001; 11: 286-294.

12. Chuang GC, Yang Z, Westbrook DG, Pompilius M, Ballinger CA, White CR, et al. Pulmonary ozone exposure induces vascular dysfunction, mitochondrial damage, and atherogenesis. ***Am J Physiol Lung Cell Mol Physiol*** 2009; 297: L209-L216.
13. Bocci V. Is it true that ozone is always toxic? The end of a dogma. ***Toxicol Appl Pharmacol*** 2006; 216: 493-504.
14. Viebahn-Hänsler R. The use of ozone in medicine. 5th ed. Iffezheim (Germany): ODREI Publishers; 2007.
15. Chang JD, Lu HS, Chang YF, Wang D. Ameliorative effect of ozone on cytokine production in mice injected with human rheumatoid arthritis synovial fibroblast cells. ***Rheumatol Int*** 2005; 26: 142-151.
16. Zamora ZB, Borrego A, Lopez OY, Delgado R, Gonzalez R, Menendez S, et al. Effects of ozone oxidative preconditioning on TNF-alpha release and antioxidant-prooxidant intracellular balance in mice during endotoxic shock. ***Mediators Inflamm*** 2005; 2005: 16-22.
17. Fuccio C, Luongo C, Capodanno P, Giordano C, Scafuro MA, Siniscalco D, et al. A single subcutaneous injection of ozone prevents allodynia and decreases the over-expression of pro-inflammatory caspases in the orbito-frontal cortex of neuropathic mice. ***Eur J Pharmacol*** 2009; 603: 42-49.
18. World Medical Association. World Medical Association Declaration of Helsinki. Ethical Principles for Medical Research Involving Human Subjects. Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964. ***Journal International de Bioéthique*** 2004; 124-129.
19. Rainsford RD. Aspirin and Related Drugs. Routledge (UK): Taylor & Francis Inc; 2004.
20. Burke JG, Watson R. Intervertebral discs which cause low back pain secrete high levels of proinflammatory mediators. ***J Bone Joint Surg Br*** 2002; 84: 196-201.
21. Latremoliere A, Woolf CJ. Central sensitization: a generator of pain hypersensitivity by central neural plasticity. ***J Pain*** 2009; 10: 895-926.
22. Brisby H. Pathology and possible mechanisms of nervous system response to disc degeneration. ***J Bone Joint Surg Am*** 2006; 88 (Suppl 2): S68-S71.
23. Cappello C, Saugel B, Huth KC, Zwergal A, Krautkramer M, Furman C, et al. Ozonized low density lipoprotein (ozLDL) inhibits NF-kappaB and IRAK-1-associated signaling. ***Arterioscler Thromb Vasc Biol*** 2007; 27: 226-232.
24. Wentworth P Jr, McDunn JE, Wentworth AD, Takeuchi C, Nieva J, Jones T, et al. Evidence for antibody-catalyzed ozone formation in bacterial killing and inflammation. ***Science*** 2002; 298: 2195-2199.