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REVIEW

Role of Intravesical Ozone in the Management of BPS/Interstitial Cystitis

Maria Verônica Pires[1] · Henrique Cunha Carvalho[2,3] · Lívia Helena Moreira[1,3,4] · Adriana Barrinha Fernandes[1,3] · Carlos José de Lima[1,3]

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Abstract

Purpose of Review In this review, studies and mechanisms of action relative to intravesical ozone in Bladder Pain Syndrome/Interstitial Cystitis (IC/BPS) will be summarized and correlated with pathologies of chronic pelvic pain in animal models and clinical trials.

Recent Findings Some studies have investigated intravesical ozone therapy in view of the disadvantages of conventional interventions and the extensive popularization of ozone in healthcare.

Summary Despite the small number of specific studies, many recent, results postulate ozone as a promising alternative for the management of IC/BPS given its antioxidant, anti-inflammatory, and immunomodulatory effect.

Keywords Ozone · Ozone therapy · BPS · Interstitial cystitis

Introduction

A Bladder Pain Syndrome/Interstitial Cystitis (IC/BPS) is a debilitating bladder disorder, defined as chronic pelvic pain associated with urinary symptoms such as frequency and/or urgency [1••, 2].

Prevalence ranged from 0.01 to 2.3% with female predominance [1••]. The RAND Interstitial Cystitis Epidemiology (RICE) postulates 3.3 to 7.9 million women in the United States aged 18 years and older have IC/BPS [3]. The pathophysiology is not fully understood, and many theories have been proposed [4]. Biochemical changes in some immune cells, including plasma and mast cells (MC), are found in the urine of the affected individuals, suggesting an association with immune dysfunction [5•, 6, 7].

The available treatments fail or become less effective over time, making it necessary to evaluate new therapeutic methods. In recent years, investigations into therapies at the molecular level, such as the nuclear erythroid factor 2-related factor 2 (Nrf2) signaling pathway, have been conducted [8•, 9]. Nrf2 has been widely studied as a regulator of cytoprotective responses and is involved in a wide spectrum of diseases, suggesting the possibility of this pathway being a common therapeutic target [10].

Ozone (O_3) is an allotrope of oxygen (O_2) naturally present in the stratosphere, composed of three oxygen atoms in a cyclic and relatively unstable structure. It was first isolated in 1839 by Christian F. Schönbein [11]. For therapeutic purposes, denominated ozone therapy, it is artificially produced by generators that conventionally promote a high voltage electrical discharge in a medical oxygen flow, producing a highly oxidant gaseous mixture with 95% O_2 and 5% O_3 [12•, 13].

There is growing scientific interest in the systemic effects and administration of ozone in various pathologies [12•, 14] and its mechanisms of action, mainly at the cellular level. The interest in ozone therapy is due to its potent

✉ Maria Verônica Pires
mariaveronicapires46@gmail.com

1 Biomedical Engineering Institute, Anhembi Morumbi University (UAM), Rua Casa do Ator, 275, São Paulo, SP 04546-001, Brazil

2 The Federal University of Technology—Paraná (UTFPR), Via Marginal Rosalina Maria dos Santos, 1233, Bloco B, Campo Mourão, PR 87301-899, Brazil

3 Center for Innovation, Technology, and Education (CITÉ), São José dos Campos Technological Park, Estrada Dr. Altino Bondensan, 500, São José dos Campos, SP 12247-016, Brazil

4 State University of Londrina (UEL), Rodovia Celso Garcia Cid, 445 Km, Campus Universitário, Londrina, PR 86057-970, Brazil

antimicrobial and anti-inflammatory action, the effect on the antioxidant defense system, and its immunomodulatory response.

The aim of this study was to conduct a review of the literature on intravesical ozone in IC/BPS, focused on the pathophysiological issues of the disease, and evidence of the possible mechanisms of ozone. The results found will be correlated with chronic pelvic pain in animal models and clinical trials, inferring the effect on Nrf2 antioxidant and nuclear factor kappa-β (NF- kβ) pro-inflammatory pathways.

Interstitial Cystitis/Bladder Pain Syndrome

IC/BPS is a heterogeneous disease, with two subtypes established and differentiated by cystoscopy [2, 4, 15, 16]. The Hunner lesion phenotype (HIC) is predominantly inflammatory with epithelial denudation, MC infiltration, and edema [1••, 2]. The prevalence varies significantly, between 3.5 and 56%, due to the cystoscopy performed in the diagnosis, in which the values are higher [16]. The non-ulcerative phenotype (NHIC) shows minor inflammatory changes [15] and overlaps with somatoform disorders [17].

Urothelial changes that interfere with cell-cell signaling through TLR7 receptor (toll-like receptor R-7) involved in immune responses [5•, 18, 19], and the somatic-visceral system activating mechanosensory and nociceptive pathways, have been discussed as fundamental mechanisms of IC/BPS [7, 15, 20].

Neurotransmitters, released by peripheral neurons, promote neurogenic inflammation and activate the degranulation of MCs [15, 21]. Degranulation of MCs promotes the release of pro-inflammatory mediators (histamine, serotonin, and tryptase), tumor necrosis factor-α (TNF-α), nerve growth factor (NGF), interleukins (IL -6, IL -8, IL -10), prostaglandin E2 (PGE2), and increase in inducible nitric oxide synthase (iNOS) [5•, 15, 22–24]. Stimulation of the TLR receptors culminates in nuclear translocation of NF-kβ, inducible transcription factor, involved in the expression of pro-inflammatory genes [25] and the regulation, activation, differentiation, and effector function of inflammatory T cells [25, 26]. The nuclear translocation of NF-kβ allows the activation of pro-inflammatory M1 macrophages, especially in the NHIC [5•]

Recently, a study evaluated the molecular mechanisms of IC/BPS via RNA sequencing (scRNA-seq) in five patients with NHIC/HIC and two unaffected controls [5•]. This study reported that fibroblasts promoted the release of inflammatory cytokines, in particular IL-6, involved in the activation of B cells, CD4, T cells, and migration of associated neutrophils to the immune system. The inflammatory mediators released act back on afferent neurons in a positive feedback loop, resulting in increased release of neuropeptides that further exacerbate the activation of degranulating MCs and inflammatory response [15, 23, 24]. These bodies of evidence suggest that manipulation of the inflammatory pathway is an interesting possibility for the treatment of IC/BPS.

Previous studies established Nrf2 as a transcription factor that regulates cytoprotective genes critical to antioxidant and anti-inflammatory responses. The investigation of the involvement of the Nrf2 pathway in bladder pathologies and that its impairment could be a factor in the progression of IC [8•] is of increasing interest. A study in an animal model of cyclophosphamide-induced IC stated that the Nrf2 pathway offered protection against bladder dysfunction by activating antioxidant genes and inhibiting oxidative stress and that IC treatments that address the Nrf2 pathway should be explored [9]. Along the same lines, a study using electron microscopy to observe the distribution of urinary exosomes in an animal model of HIC observed that Nrf2 expression was negatively regulated and that its levels were inversely related to MEG3 (maternally expressed 3), an IncRNA used as a biomarker of bladder diseases [19]. Similarly, Wang et al. [8•] observed that negative regulation of MEG3 was related to reduction of the inflammatory process, positive regulation of Nrf2, and inhibition of the p38/NF-κβ pathway [8•].

Ozone Therapy and Molecular Mechanisms of Ozone

In biological systems, ozone administration instantly reacts with the macromolecules of cell membranes, including lipids, among them polyunsaturated fatty acid (PUFA), glycoproteins, amino acids (mainly aromatic), and DNA [12••], according to the mechanism proposed by Criegee [27]. The reactions promote formation of two fundamental messengers, hydrogen peroxide from ozone (H_2O_2) and 4-hydroxynonenal (4HNE), related to lipid oxidation product (LOP) [12••, 13, 28, 29]. The LOPs are long-lasting late messengers that diffuse into all cells and are involved as signal transduction molecules [12••, 13]. Membrane-associated H_2O_2 is an early, short-lived messenger that acts via cysteine residues and/or reduction via glutathione (GSH) assuming regulation of antioxidants, SOD demand (superoxide dismutase), and CAT (catalase) as in oxidative stress processes [12••, 13, 30].

Clinical and experimental evidence has suggested that the therapeutic effects of ozone therapy are related to mild and dose-dependent antioxidant response (hormesis concept) through modulation of the Nrf2 pathway, and immunomodulatory pathway via NF-κβ [30•, 31–34].

Ozone in the Modulation of Nrf2 and NF-κB Pathways

Nrf2 is a gene expression regulator controlled by an antioxidant response element (ARE). Under basal conditions, Nrf2

binds to its repressor Keap1 (Kelch-like ECH-associated protein (1) creating an inactive complex in the intracellular environment that promotes rapid degradation [10, 13, 34]. NF-κβ regulation by ozone H_2O_2 probably occurs through the modulation of anti-inflammatory cytokines, acting as fine-tuning signaling molecules [35]. Furthermore, H_2O_2 can act as a promoter of the Nrf2 pathway, by increased expression of casein kinase 2 (CPK 2) and inhibition of the signaling pathway MAPK (mitogen-activated protein kinase) modulating oxidative stress and apoptosis [13, 32]. MAPK inhibition suppresses NF-κβ activation reducing inflammatory molecules such as COX-2 and iNOS that contribute to the progression of inflammatory diseases [36].

The 4HNE, induced by ozone, modifies cysteine residues (S-HNE or S——S) of Keap1 with dissociation of Nrf2 to Keap1, followed by nuclear transfer and accumulation of Nrf2. In the nucleus, Nrf2 binds to proteins in the muscle aponeurosis fibromatosis (Maf) promoting transcription of the ROS in DNA in different genes involved in the antioxidant response. These genes encode proteins involved in protein-homeostasis, response to oxidative stress, DNA repair, proliferation, biogenesis, and mitochondrial function among others [37]. This control of oxidative stress is linked to the transcription of antioxidant enzymes such as heme oxygenase 1 (HO-1), SOD, glutathione peroxidase (GPx), glutathione-S-transferase (GST), CAT, GSH-reductase (GR), NAD(P)H dehydrogenase [quinone] 1 (NQO1), heat shock proteins (HSPs), and cytochrome P450 (CYP450) enzymes [12••, 13, 34].

Inflammatory modulation by Nrf2 occurs through the regulation of redox homeostasis and suppression of pro-inflammatory genes [32, 34]. HO-1, directly and indirectly, inhibits pro-inflammatory cytokines such as TNFα, INFγ, IL1β, IL6, IL8, and pro-inflammatory genes such as cyclooxygenase-2 (COX-2) and activates anti-inflammatory cytokines [32]. The indirect action is related to the degradation of heme into carbon monoxide (CO) and iron. In this sense, CO acts as an inhibitor of the NF-κβ signaling pathway, determining a reduction in the expression of inflammatory cytokines, suppressing the activation of macrophages, and interfering with pain signaling by suppressing the spinal glutamate receptor 6 (GluR6NFκB/p65) [38, 39].

Ozone Administration

The therapeutic effects of ozone therapy are dose-dependent, according to the hormesis concept, in which low doses are highly effective, and act as a bioregulator [12••, 30•, 31, 40•]. Different clinical trials have shown the efficacy of ozone in various pathologies, especially in chronic inflammatory diseases. These pathologies involve high oxidative stress mediated by ROS, impaired antioxidant capacity, and immune imbalance. Ozone concentrations of 10–50 µg/mL determined at a total ozone dose of 5 to 6 mg per treatment [30•, 31] showed physiologically effective responses in systemic applications. Administration of treatments with ozone concentrations equal to or greater than 60 µg/mL (60–100 µg/mL) is restricted to the topical application [29, 30•].

Clinical trials in ozone wound healing, both acute and chronic, described an increase in cell proliferation and expression of PDGF, TGF-β1, and VEGF, with effects on epithelialization and neovascularization [41–43]. These effects were linked to the potential for inducing controlled oxidative stress, immunomodulatory, and neuroprotective effects with changes in neural signaling and the formation of free radicals [32, 42, 44, 45]. The benefits of ozone therapy in the treatment of neuropathic pain, hyperalgesia, allodynia, and fibromyalgia, among others, associated with antioxidant and anti-inflammatory action have also been reported [42, 43, 46–48].

Moreover, ozone therapy has been effective in renal pathologies. Some studies using ozone before or after ischemia-reperfusion processes have demonstrated a protective effect, positive regulation of antioxidant enzymes, and a reduction in apoptosis rates [49–51]. The benefits observed in protocols of the O_2+O_3 mixture could also be justified by action on the Nrf2 pathway. Ozone promotes cell survival and increases cell proliferation by blocking apoptotic processes, particularly by reducing the expression of caspase 1-3-9, hypoxia-inducible factor α (HIFα), TNF-α among others [51].

Ozone in Pelvic Pathologies

The Multidisciplinary Approach to The Study of Chronic Pelvic Pain (MAPP) and the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) investigated the two most prevalent chronic urological pain disorders: IC/BPS and chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS). When these observations associated with the therapeutic complexity of pelvic pathologies were correlated, they encouraged the verification of ozone therapy response in pelvic pathologies associated with chronic pain [18, 52].

The use of ozone therapy in complications of the treatment of pelvic tumors has been reported. In a recent review, Clavo et al. [53•] address potential mechanisms of ozone action in CPPS induced by radiotherapy and chemotherapy. According to the authors, the benefits that ozone—in adequate doses—could induce an adaptive oxidative response and that probably, the main effect of ozone therapy is to reset the NF-κB/Nrf2 balance. The use of rectal insufflations of ozone and topical ozonated oil was described by Clavo et al. [54] in patients with hemorrhagic proctitis as a complication of radiotherapy in prostate or uterine tumors. Ozone concentrations between 10 and 30 µg/mL induced a

positive effect against rectal bleeding, without serious side effects. The authors attributed these results to modulation of the inflammatory response by the suppression of NF-κβ, associated with the topical effect of ozonated oil by the production of H_2O_2, thereby improving wound healing with the production of trophic factors and angiogenesis.

Katibov and Alibekov [55] described the positive effects of the association between transrectal ozone and magnetic therapy in 142 patients with chronic bacterial prostatitis confirmed by the NIH-CPSI questionnaire, prostate volume, voiding frequency, and microbiological parameters.

Ozone in Bladder Pathologies

Animal Model

In animal models, ozone has been shown to be a beneficial agent for intravesical application in the treatment of various IC conditions.

Studies describe the immuno-histopathological and oxidative-antioxidative effects of intravesical ozonized saline. Teke et al. [56] evaluated the immunohistopathological and oxidative-antioxidative effects of intravesical ozonated saline at a concentration of 20–25 µg/mL in rats with bladder cancer, by means of the oxidants malondialdehyde (MDA), NO and myeloperoxidase (MPO), and antioxidants SOD and GSH. The authors reported the absence of neoplastic proliferation (normal urothelium) in 16.7% of the histological samples from the group treated with ozone and a lower number of high-grade lesions among those with and without ozone treatment (8.3 and 36.4% respectively). The oxidant-antioxidant systems were balanced and showed no evidence of histopathological tissue alterations. Observations regarding the effect of ozone on oxidative/nitrosative stress were consistent with data from Tasdemir et al. [57] who evaluated an animal model of bacterial cystitis caused by *Escherichia coli.* They found a reduction in MDA, NO (nitric oxide), and MPO in the group treated with ozonated saline infusion (20–25 µg/mL, three days). The elevation of SOD and GPx was associated with alterations in epithelial histopathology. The authors also observed the absence of bacterial translocation in the ozone group.

The impact of ozone therapy in animal models of IC has been reported. Bayrak et al. (2014) [58] studied an animal model of hydrochloric acid (HCL)-induced IC. The treated groups received 10 mL of intravesical infusion of ozonated saline solution at a concentration of 20–25 µg/mL, two sessions per week, last 10 minutes. Assessments were carried out over three weeks (6 sessions) for early effects and six weeks (12 sessions) for late effects. Histology showed a significant decrease in leukocyte and MC counts and basement membrane integrity compared to the control group. The changes were more visible in the group that received 12 sessions, and the results showed the anti-inflammatory effect of ozone. Tasdemir et al. (2013b) [59], also in an animal model of hemorrhagic IC induced by cyclophosphamide (CP), evaluated the use of 0.9% saline solution containing 20–25 µg/mL of ozone, for three days, before and after administration of CP. Results demonstrated prevention of CP-induced urothelial damage associated with reduced oxidative stress, inflammation, and NO levels.

Clinical Ozone

The clinical use of ozone in bladder pathologies has been investigated and has not only shown good clinical results, but the absence of side effects as well.

An interesting case report by Clavo et al. (2005) [60] describes results from bladder instillations (eight weeks, three sessions a week) with ozonated bidistilled water (concentration of 20–25 µg/mL, infusion time of 30 minutes) in patients with hematuria associated with radiation cystitis. In 2020, the authors described the use of the O_2+O_3 mixture or instillations of ozonated water into the bladder in two patients with severe pelvic pain and bladder symptoms associated with radiotherapy or chemotherapy, with no response to treatments established by the American Urological Association (AUA). One patient did not improve and was referred for cystectomy. The authors recommended the use of ozone with a frequency of two to three weekly sessions, mainly in patients with chronic conditions refractory to conventional treatments [61]

The benefits of using ozone in bladder infectious pathologies have been reported in several studies. Daniluk et al. (2000) [62] used the intravesical O2+O3 mixture in 72 individuals (65 females and 7 males) with bacterial cystitis for 18 months, with the number of sessions ranging between 10 and 18, according to the bacteriological exam. Patients were evaluated for symptoms of pain, burning, and urine cultures with tests were performed at time intervals of three and six months after ozone therapy. In all women, there was control of clinical symptoms, with a recurrence of 6% in six months. Among men, 86% were asymptomatic and bacteriologically negative after six months. Muzi et al. [63] in a group of eight patients with chronic cystitis, four with associated *E. coli* infection, used ozone autohemotherapy (30–40 µg/mL, once a week) and bladder insufflation of 100 mL of ozone in a concentration of 30 µg/mL, twice a week. All patients showed progressive improvement in urinary symptoms, control of the infectious condition, and absence of trigonitis after two months. These results were corroborated in the study of Yuldashev et al. [64] who investigated the effectiveness of ozone therapy in 50 patients with acute bacterial cystitis.

Microcirculation parameters and pathomorphological changes in the bladder mucosa of patients with IC undergoing ozone therapy were described by Neimark et al. (2014) [65] In this study, one group (30 patients) was submitted to vesical instillations of ozonated saline solution with a concentration of 2–4 µg/mL. A control group (20 patients) received bladder instillations that contained multiple components (procaine, syntomycin, dimexide, diphenhydramine, analgin solution). A third group (15 patients) received parenteral infusions of ozonated saline (1 µg/mL). Histological evaluation showed structural reorganization of the bladder mucosa with reduction in the inflammatory process (80% hyperemia and 64% edema) and intensification of reparative processes, with urothelial proliferation and urodynamic improvement in the group submitted to ozone. Changes were more consistent in the group that received intravesical administration.

Recently, Pires et al. [66••] in a clinical trial IC/BPS study, using a mixture of O_2+O_3 (concentration of 41 µg/mL, infusion of 60 mL of gas, with an ozone mass of 2.5 mg). The group of 16 patients was refractory to at least two treatments established by the AUA. The sessions were conducted twice a week, totaling six sessions, with the results evaluated by means of the ICSI/ICPI Score (O'Leary–Sant Questionnaire). On conclusion of the protocol, in the ICSI/ICPI score values, and after 180 days, the values reached a reduction of 92.3%. Approximately 25% showed a 47% reduction in scores at the end of treatment, and a 95.1% reduction at the end of the follow-up period. At the end of 180 days, 81.2% of patients had no urgency and 75% had no pelvic pain. The results observed suggested a progressive effect of intravesical ozone, not only related to the therapeutic period but to a phenomenon also observed by Clavo et al. [53], who administered rectal insufflations in the treatment of pelvic pain associated with radiotherapy and chemotherapy.

The patients in the study of Pires et al. [66••] continued to be followed-up after 180 days (unpublished data). In 18.7% (3) patients there was a single episode of recurrence of bladder symptoms, for ICSI values of urgency, in the presence of gynecological infection. The scores were lower than those observed on admission. One patient underwent the therapeutic protocol previously described. After the event, no recurrence was observed in the follow-up of longer than one year. In 75% of (12) patients with a minimum follow-up of 24 months, there was no recurrence of IC/BPS. One patient (6.25%) abandoned follow-up after the first year, with no report of recurrence.

Discussion

At present, knowledge about the pathogenic mechanism of IC/BPS has shown the role of afferent hypersensitivity with neurogenic inflammation [15], and immune activation with clonal expansion of B cells [5•] and changes in iNOS [22]. This issue allows us to infer that in the process of IC/BPS, there is manipulation of the inflammatory pathway and this is an interesting pathway leading to therapeutic management of IC/BPS.

Evidence regarding the involvement of the Nrf2 pathway in the progression of IC associated with the observation that its upregulation was correlated with a reduction in the inflammatory process [8•] encouraged the evaluation of therapeutic methods that address the Nrf2/NF-κB pathway in the treatment of IC/BPS.

The therapeutic effects of ozone therapy have been attributed to a mild, dose-dependent antioxidant response with modulation of the Nrf2/NF-κB pathway [13, 30•, 31–33] which makes its use feasible in the treatment of chronic pelvic pain and consequently in the treatment of IC/BPS.

Nrf2 has been extensively involved in cytoprotective regulation by means of the upregulation of antioxidant genes, suppression of inflammatory genes related to the release of pro-inflammatory cytokines, inhibition of oxidative stress, and suppression of mechanisms related to chronic pain [9, 67–70].

Studies in animal models using ozone via vesical instillations of ozonated solutions or by infusion of the O_2+O_3 mixture have demonstrated reduction in the oxidative and inflammatory stress markers, including NO, with significant reduction in infiltration of inflammatory cells and resolution of edema and vascular congestion [56–59].

Recent evaluations related to the use of intravesical ozone, with the aim of controlling the clinical symptoms of IC/BPS in patient's refractory to conventional treatments, were investigated by Pires et al. [66••] and Clavo et al. [53•]. A progressive response that was not associated with the treatment period only was demonstrated. This was probably represented by the observations of Neimark et al. [65], who found that the urothelial reparative process was developed in a period of approximately 14 days.

A better understanding is needed regarding the concentration (dose), volume, number of sessions, time of exposure, and administration of intravesical ozone. We agree with Clavo et al. [53•], relative to the performance of two to three weekly sessions, especially in highly symptomatic patients and those with prolonged evolution of the disease. However, the ideal concentration (therapeutic window) for use in cases of IC/BPS is still open. The use of ozone concentrations between 30 and 40 µg/mL with anti-inflammatory, immunomodulatory, and antioxidant-stimulatory effects appeared to show more effective results in refractory patients [63, 66••].

Conclusions

The use of intravesical ozone is a promising alternative for the treatment of IC/BPS, based on the therapeutic effect that probably modulates the Nfr2 and NF-κβ pathways. Studies in animal models and clinical trials are still incipient and recent, and randomized studies with the aim of establishing effective concentrations should be conducted to investigate therapeutic protocols that will reveal ozone as another alternative for the management of IC/BPS.

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Declarations

Conflict of Interest The authors declare no competing interests.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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