Systematic Review

Oxygen–Ozone Therapy for the Treatment of Knee Osteoarthritis: A Systematic Review of Randomized Controlled Trials

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Purpose: To review the available literature on the application of oxygen–ozone therapy (OOT) in the treatment of knee osteoarthritis (KOA) to understand its therapeutic potential and to compare it with other conservative treatment options. **Methods:** A systematic review of the literature was performed on the PubMed, Cochrane, Embase, ResearchGate, and PedRo Databases, with the following inclusion criteria: (1) randomized controlled trials (RCTs), (2) written in English, (3) published on indexed journals in the last 20 years (1998-2018), (4) dealing with the use of ozone intra-articular injection for the treatment of KOA. The risk of bias was assessed by the Cochrane Risk of Bias tool for RCTs. **Results:** Eleven studies involving 858 patients in total (629 female and 229 male) were included. Patients in the control groups received different treatments: placebo in 1 trial; hyaluronic acid in 2 studies; hyaluronic acid and PRP in 1 trial; corticosteroids in 4; and hypertonic dextrose, radiofrequency, or celecoxib + glucosamine in the remaining 3 trials. In looking at the quality of the available literature, we found that none of the studies included reached "good quality" standard, 2 were ranked as "fair," and the rest were considered "poor." No major complications or serious adverse events were reported following intra-articular OOT, which provided encouraging pain relief at short term. On the basis of the available data, no clear indication emerged from the comparison of OOT with other established treatments for KOA. **Conclusions:** The analysis of the available RCTs on OOT for KOA revealed poor methodologic quality, with most studies flawed by relevant bias, thus severely limiting the possibility of drawing conclusions on the efficacy of OOT compared with other treatments. On the basis of the data available, OOT has, however, proven to be a safe approach with encouraging effects in pain control and functional recovery in the short-middle term. **Level of Evidence:** Systematic review of Level I and III studies.

Osteoarthritis (OA) is a degenerative condition that causes pain, impaired function, and affects daily activities.[1] OA is associated with great morbidity and low mortality, which makes it an extremely frequent, chronic disabling disease, especially in older Western populations.[2] Knee osteoarthritis (KOA) is associated with chronic inflammation that causes persistent oxidative damage, which subsequently leads to joint degeneration.[3] Chronic oxidative stress plays an important role in KOA, so the suppression of oxidative damage without disruption of the antioxidant defense network could be an important therapy target.[3] Treatment options for painful KOA are often unsatisfactory, as represented by 40% of patients reporting persisting postoperative pain following total knee arthroplasty.[4] There are no currently approved KOA treatments capable of slowing OA-related structural progression,[5] so the main goals of the conservative treatment are to provide symptomatic relief, improve joint function, and delay surgical intervention.

One of the main actions of intra-articular treatments, ranging from corticosteroids to hyaluronic acid (HA) and biologic products, is to reduce inflammatory distress within the joint.[6,7] In recent years, there has been a growing interest in the effects of ozone,[8] which can be safely delivered intra-articularly and whose use

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is in constant increase in an outpatient setting due to the ease of preparation methods. Ozone (O_3), a gas discovered in the mid-19th century, is a molecule consisting of 3 atoms of oxygen in a dynamically unstable structure due to the presence of mesomeric states.[9] Intra-articular administration of an adequate mixture of oxygen–ozone is supposed to reduce pain, to have protective immunomodulatory effects on cartilage, and to reduce oxidative stress, thus potentially representing an alternative to other injective methods[10]; several researchers worldwide and many years of clinical experience have indicated that O_3 has the capacity to modulate inflammation.[2] It is highly reactive and, when injected into a joint capsule, it is able to stimulate fibroblastic joint repair, reduce inflammation, and may promote new cartilage growth.[2] O_3 produces acute oxidative stress with a paradoxical antioxidant effect: it has been shown that the controlled administration of O_3 may promote an oxidative preconditioning or adaptation to oxidative stress that in turn stimulates the antioxidant endogenous system, finally resulting in a protective state against tissue damage.[11] When injected into the knee, O_3 is mixed with oxygen (O_2) and dissolves into the synovial fluid, which contains antioxidants and proteins, and generates reactive oxygen species and lipid oxidation products. These molecules inactivate and inhibit proteolytic enzymes, decrease the release of proinflammatory cytokines, induce the proliferation of chondrocytes and fibroblasts, and promote the synthesis of antioxidant enzymes and immunosuppressive cytokines.[3] All these processes counteract the proinflammatory and pro-oxidative circuit that arises in KOA, resulting also in an increase in tissue oxygen supply through a hemorheologic action based on vasodilatation and angiogenesis stimulation.[11] In light of these biologic actions, many patients affected by KOA are being treated by oxygen–ozone therapy (OOT), but there is still a lack of consensus on the efficacy of this treatment.

Our hypothesis was that OOT could be considered as an effective treatment in terms of pain relief and improving joint function, with results comparable with those of other traditional therapeutic approaches. The purpose of the present paper is to review the available literature on the application of OOT in the treatment of KOA to understand its therapeutic potential and to compare it with other conservative treatment options.

Materials and Methods

A systematic review of the literature was performed on the use of intra-articular injections of oxygen–ozone in KOA as a conservative approach to reduce pain and improve joint function. A search was conducted for English articles published up to the end of May 2018. The electronic databases PubMed, Cochrane Library, Embase, and PEDro were investigated, using the following formula:

(ozone therapy OR ozone injection OR ozone) AND (knee OR intra − articular OR osteoarthritis)

Furthermore, the ResearchGate database was investigated using the following key words: "ozone" AND "knee." Database-searching was supplemented by screening reference lists and tracking citations included in trials to identify additional studies. The screening process and analysis was conducted separately by 2 independent observers (an orthopaedic surgeon [F.V.], and a physiatrist [C.S.]). First, the articles were screened by title and abstract.

The following inclusion criteria for relevant articles were used during the screening: (1) randomized controlled trials (RCTs) on humans, (2) written in English, (3) published in indexed journals in the last 20 years (1998-2018), and (4) dealing with the use of intra-articular O_3 injections for the treatment of KOA. Exclusion criteria were articles written in other languages, animal and in vitro trials, reviews, nonrandomized studies, or trials analyzing other applications of O_3 not related to KOA.

In the second step, the full texts of the selected articles were screened, with further exclusions according to the previously described criteria. A flowchart of the systematic review is provided in Figure 1. Relevant data were then extracted and collated in a unique database, with the consensus of the 2 observers, to be analyzed for the purposes of the present manuscript. The risk of bias was assessed using the Cochrane Risk of Bias tool for Randomized Controlled Trials, which evaluates 7 different type of bias. Each of them, based on specific criteria, was classified "low risk," "high risk," or "unclear risk." Subsequently, the results of this assessment were converted to Agency for Healthcare Research and Quality standards, which ultimately rank the RCTs in "good quality," "fair quality," and "poor quality." The level of evidence of each article was also re-evaluated according to the criteria established by Hohmann et al.[12]

Results

A total of 11 studies published from 2011 to May 2018 dealing with O_3 injection outcomes in the treatment of KOA were ultimately included in this review (Fig 1). A detailed description of each study has been provided in Table 1.[8,13-22]

Study Design and Quality

All studies were, as per inclusion criteria, RCTs. The study designs were highly variable: patients in the control groups received different injection or treatment, such as HA in 3 studies; corticosteroids in 4 studies; and placebo, hypertonic dextrose, radiofrequency, or celecoxib + glucosamine in the remaining 4 studies.

In looking at the quality of the available literature by AHQR standard, we found that none of the studies

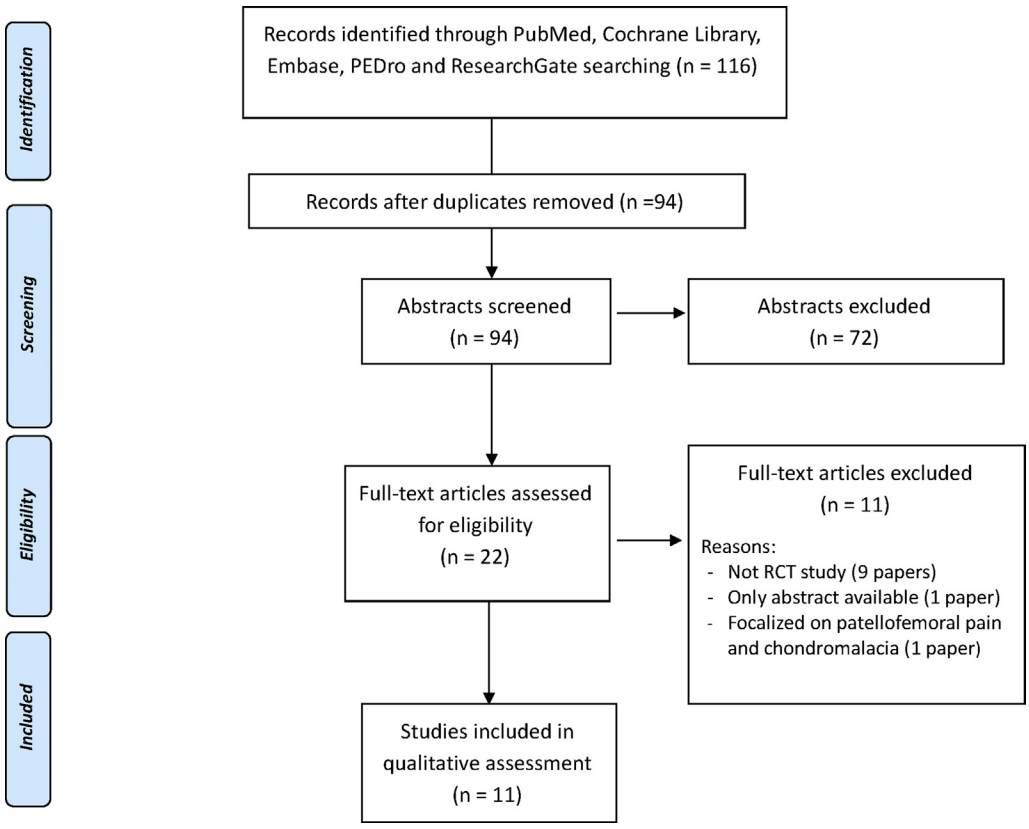

Fig 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses flowchart resuming the papers' selection process.

included reached a "good quality" standard, 2 were ranked as "fair quality" RCT, and the rest were considered as "poor quality." The results of the analysis performed with the Cochrane Risk of Bias tool for RCT are detailed in Table 2. In regards to the random sequence generation process, it was not specified in 3 papers, whereas it was based on odd/even number in the study of Chansoria et al.[21] The method of allocation concealment was described in nearly none of the studies included, except for 2.[13,14] Four papers reported outcomes incompletely.[15-18] Regarding sample size calculation, in all the included trials the power analysis methods were not fully clarified. Three trials were double blinded, 2 were single blinded, and the others were unblinded. Moreover, the risk of attrition bias was unclear for the majority of the studies: in most cases, it was not specified how many patients were screened, how many were excluded from randomization or why, how many were lost to follow-up, and for which specific reasons. Flow diagrams depicting the patient selection process were reported only in 4 papers.[13,14,19,20] Finally, we found that 3 trials were prospectively registered in a public clinical trial registry,[13,14,16] one was registered retrospectively,[19] whereas the others did not mention any registration, which should be mandatory according to the Consolidated Standards of Reporting Trials 2010 guidelines.

Patients and Evaluation Methods

Eleven studies involving 858 patients in total (629 female and 229 male) with KOA were included in this review. Mean age was 61 years. All papers except one[15] performed a radiographic assessment before treatment and classified patients according to Kellgren-Lawrence (K-L) grading system. Five papers included patients with radiographic K-L grades II-III,[13,16,17,19,20] 4 papers included patients with radiographic K-L grades I-II,[8,18,21,22] whereas Babaei-Ghazani et al.[14] included grades I-III.

Baseline and follow-up assessments were based solely on clinical scores in all studies except for Hashemi et al.,[15] who also measured inflammatory cytokines (interleukin-1b and tumor necrosis factor-alpha) serum levels, and Babaei-Ghazani et al.,[14] who performed ultrasound examinations to evaluate joint effusion. Visual analog scale (VAS) and Western Ontario and McMaster Universities Arthritis Index (WOMAC) scores were the most commonly used. In only 2 cases, the VAS was replaced by the Numerical Rating Scale and the MacNab score, whereas in 4 studies functional outcome

Table 1. Data Extracted From Included Studies

Publication	Study Design	Score	Patients Features	Ozone-Preparation Method	Therapeutic Protocol and Evaluations	Final FU, mo	Results	Overall Performance	Level of Evidence
Babaei-Ghazani et al.,[14] 2018	Double-blind RCT (ozone vs triamcinolone 40 mg)	WOMAC, VAS, ROM, effusion on US images	62 (31 vs 31) Age: 59.7 vs 56.3 y Sex: 28 F: 3 M vs 24 F: 7 M K-L: I-III	Machine: NA V and Conc.: 10 mL of 15 μg/mL	Single injection FU at 1 week, 1 and 3 mo	3	Both steroid and ozone injections are effective in terms of pain relief. Steroid shows an earlier improvement, whereas ozone effect seems to be persistent and last longer.	=	I
Raeissadat et al.,[19] 2018	Double- blind RCT (ozone vs HA)	VAS and Persian version of the WOMAC	141 (67 vs 74) Age: 58.1 vs 61.1 y Sex: 50 F:17 M vs 56 F:18 M K-L: II-III	Machine: Ozomed smartline (Kastner-Praxisbedarf GmbH) V and Conc.: 10 mL of 30 μg/mL	Weekly for 3 wk Single FU session at 6 mo	6	Ozone and HA improve VAS and WOMAC score. No statistical difference between the 2 groups	=	I
Lopes De Jesus et al.,[13] 2017	Double-blind RCT (ozone vs placebo)	VAS, Lequesne Index, TUG test, SF-36, WOMAC and Geriatric Pain Measure	96 (61 vs 35) Age: 70.5 vs 69.5 y Sex: 56 F:5 M vs 30 F:5 F K-L: II-III	Machine: Ozone & Life (O&L) 3.0 RM generator V and Conc.: 10 mL of 20 μg/mL	Weekly for 8 wk FU at 4-8-16 wk	4	Significant reduction in pain compared with placebo. Controversial results in joint function and quality of life.	+	I
Feng and Beiping[22] 2017	RCT (ozone + celecoxib + glucosamine vs celecoxib + glucosamine)	VAS and Lysholm score	76 (35 vs 41) Age: 64.6 vs 62.3 y Sex: 20 F:15 M vs 23 F:18 M K-L: I-II	Machine: NA V and Conc.: 20 mL of 20 μg/mL	Twice a week for 6 wk FU at 1-3-6 wk	1,5	VAS score improved significantly compared with the control group ($P < .05$) only at 3-wk FU. Lysholm score increased significantly at 1 and 3 wk. No statistically significant difference in VAS and Lysholm scores at 6 wk between the 2 groups	+	III

(continued)

Table 1. Continued

Publication	Study Design	Score	Patients Features	Ozone-Preparation Method	Therapeutic Protocol and Evaluations	Final FU, mo	Results	Overall Performance	Level of Evidence
Duymus et al.,[20] SSTA 2017	RCT (ozone vs HA vs PRP)	VAS and WOMAC score	102 (33 vs 34 vs 35) Age: 60.4 vs 60.3 vs 59.4 y Sex: 32 F:1 M vs 33 F:1 M vs 31 F:4 M K-L: II-III	Machine: NA V and Conc.: 15 mL of 30 μg/mL	Four injections in 1 wk. FU at 1, 3, 6, and 12 wk	3	The ozone was effective for only the first 3 mo. Both PRP and HA were superior to the ozone group.	−	III
Hashemi et al.,[8] 2015	RCT (ozone vs hypertonic dextrose)	VAS and WOMAC score	80 (40 vs 40) Age: 59.1 vs 57.3 y Sex: 23 F:17 M vs 26 F:14 M K-L: I-II	Machine: NA V and Conc.: 5-7 mL of 15 μg/mL	Three injections with 7-10 days' interval FU at 3 mo	3	Hypertonic dextrose or ozone significantly decrease pain and improve functional status without any significant difference between the 2 groups in the outcome.	=	III
Hashemi et al.,[15] 2017	Blind RCT (ozone vs triamcinolone 50 mg)	NRS and Oswestry Disability Index IL-1b and TNF-a serum level	61 (30 vs 31) Age: 56.7 vs 54.8 y Sex: 19 F:11 M vs 20 F:11 M K-L: NA	Machine: NA V and Conc.: 5 mL of 35 μg/mL	Single injection FU at 1, 3, and 6 mo	6	NRS and ODI were not significantly different at 1 mo but were different at 3 and 6 mo in favor of the ozone group. In addition, inflammatory cytokines were significantly lower in the ozone group at 3 and 6 mo.	+	III
Hashemi et al.,[16] 2016	RCT (ozone vs radiofrequency)	VAS and OKS	72 (36 vs 36) Age: 66.7 vs 68.3 Sex: 31 F:5 M vs 28 F:8 M K-L: II-III	Machine: Ozone Generator HERRMANN V and Conc.: 10 ml of 40 μg/ml + 5 ml of 10 μg/ml periarticular	3 times during the first week, twice during the second and once a week for further 3 wk. Single f-up at 12 wk.	3	No statistically significant differences were found between the 2 groups, except for patients older than 65 y (radiofrequency resulted in a superior improvement of OKS [$P = .0001$])	=	III

(continued)

Table 1. Continued

Publication	Study Design	Score	Patients Features	Ozone-Preparation Method	Therapeutic Protocol and Evaluations	Final FU, mo	Results	Overall Performance	Level of Evidence
Invernizzi et al.,[17] 2016	Blind RCT (Ozone vs HA)	VAS, OKQ, SF-12, and EuroQoL	42 (22 vs 20) Age: 70.3 vs 70.7 y Sex: 16 F:6 M vs 13 F:7 M K-L: II-III	Machine: Ozonline E80 generator (Eco3 s.n.c.) V and Conc.: 20 μg/mL	Once a week for 4 wk Final FU 4 wk after last injection	1	Pain was reduced ($P < .01$); the only differences in VAS scores among the 2 groups emerged at the final FU with a statistically significant better scores in HA group.	=	III
Chansoria et al.,[21] 2016	RCT (ozone + LA vs ozone + CS + LA)	VAS and WOMAC	80 (40 vs 40) Age: 59 vs 57 y Sex: 22 F:18 M vs 24 F:16 M K-L: I-II	Machine: NA V and Conc.: 10 mL of 20 μg/mL	Single injection FU at 1, 3, and 6 mo	6	Significant pain relief and function improvement at 1 month in both groups. At 6 mo, VAS and WOMAC scores improved significantly more for the ozone + CS group than the other.	NA	III
Mishra et al.,[18] 2011	Double-blind RCT (ozone + LA vs CS + LA) with cross-over	Overall satisfaction, Modified MacNab Method, WOMAC	46 (23 vs 23) Age: 42 y Sex: 24 F:22 M K-L: I-II	Machine: NA V and Conc.: 10 mL of 30 μg/mL	Once a month for 3 mo. FU at 3 and 6 mo after first injection	3	Success rate in the ozone group was 80% at 3 mo and 90% at 6 mo. In the other group, patients response rate was 60%, but peak up to 91% at 6 mo after cross-over	+	II

NOTE. OOT for the treatment of knee osteoarthritis: data extracted from the 11 RCTs included in the review (+, =, and − signs reflect the overall performance of OOT compared with the control group(s) of any study analyzed).

Conc., concentration; CS, corticosteroids; F, female; FU, follow-up; HA, hyaluronic acid; K-L, Kellgren-Lawrence; IL-1B, interleukin 1B; LA, local anesthetic; M, male; NA, not available; NRS, Numerical Rating Scale; ODI, Oswestry Disability Index; OKQ, Oxford Knee Questionnaire; OKS, Oxford Knee Score; OOT, oxygen−ozone therapy; PRP, platelet-rich plasma; RCT, randomized controlled trial; ROM, range of motion; SF-36, Short-Form 36; TNF-a, tumor necrosis factor alpha; TUG, Timed Up and Go; US, ultrasound; V, volume; VAS, visual analog scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

Table 2. Quality Assessment of Included Studies

Publication	Random Sequence Generation	Allocation Concealment	Selective Reporting	Other Bias	Blinding of Participants and Personnel	Blinding of Outcome Assessment	Incomplete Outcome Data	AHRQ Standards
Babaei-Ghazani et al.,[14] 2018	Low	Low	Low	Unclear	Low	Low	Low	Fair
Raeissadat et al.,[19] 2018	Low	Unclear	Low	Unclear	Low	Low	Low	Poor
Lopes De Jesus et al.,[13] 2017	Low	Low	Low	Unclear	Low	Low	Low	Fair
Feng and Beiping[22] 2017	Low	Unclear	Low	Unclear	High	High	Unclear	Poor
Duymus et al.,[20] 2017	Low	Unclear	Low	Unclear	High	High	Low	Poor
Hashemi et al.,[8] 2015	Unclear	Unclear	Low	High	High	High	Unclear	Poor
Hashemi et al.,[15] 2017	Low	Unclear	High	Unclear	High	Low	Unclear	Poor
Hashemi et al.,[16] 2016	Unclear	Unclear	High	Unclear	High	High	Unclear	Poor
Invernizzi et al.,[17] 2016	Low	Unclear	High	High	High	Low	Unclear	Poor
Chansoria et al.,[21] 2016	High	Unclear	Low	High	High	High	Unclear	Poor
Mishra et al.,[18] 2011	Unclear	Unclear	High	High	Unclear	Unclear	Unclear	Poor

NOTE. Quality assessment of included studies with Cochrane Risk of Bias tool for Randomized Controlled Trials and conversion to Agency for Healthcare Research and Quality (AHRQ) Standards:

"Good quality": All criteria met (i.e., low for each domain); "Fair quality": One criterion not met (i.e., high risk of bias for one domain) or 2 criteria unclear, and the assessment that this was unlikely to have biased the outcome, and there is no known important limitation that could invalidate the results; "Poor quality": One criterion not met (i.e., high risk of bias for one domain) or 2 criteria unclear, and the assessment that this was likely to have biased the outcome, and there are important limitations that could invalidate the results; Poor quality: Two or more criteria listed as high or unclear risk of bias.

was expressed without the help of WOMAC but by the use of Oswestry Disability Index, Lysholm scores and, in 2 studies, the Oxford Knee Questionnaire.

Treatment

Concerning the preparation method of the injected gas, several different machines were tested: 4 authors specifically reported the device adopted for O_3 production[13,16,17,19] (Table 1), whereas the rest of the authors did not mention the equipment adopted. In addition, volume and concentration of injected O_3 was inconsistent, ranging from 5 to 20 mL of injected volume and from 15 to 40 μg/mL of concentration (Table 1). Regarding delivery methods of the O_3, it was intra-articularly injected in all studies. One paper investigated the effect of a concurrent intra- and peri-articular injection.[16] Therapeutic protocols were rather different in terms of number of injections and their frequency.

Complications

No major complications or serious adverse events were reported in any of the trials included in the present review.

Reported Clinical Outcome

Lopes de Jesus et al.[13] conducted the only placebo-controlled RCT, and they showed a significantly greater efficacy of O_3 in pain relief, joint function, and quality of life compared with the control group. Mishra et al.[18] found a greater success rate in the O_3 group than in those who received a corticosteroid injection. Hashemi et al.,[15] also confirmed a significant better outcome of the O_3 group compared with those who received a corticosteroid injection at 3 and 6 months' follow-up, as well as Babaei-Ghanazi et al.,[14] who suggest that, although steroid injection showed an earlier improvement, O_3 seemed to be more persistent and have a longer-lasting effect. In contrast, Hashemi et al.[8] showed no significant difference neither between O_3 and hypertonic dextrose injection nor between O_3 and radiofrequency (with the exception of patients older than 65 years).[16]

In Feng and Beiping's trial,[22] results were controversial: pain improved significantly more in the O_3 + celecoxib group than in the celecoxib group only at 3 weeks' follow-up. The paper by Chansoria et al.[21] also presented controversial findings, because O_3 was injected both in the treatment and control group (with or without concurrent intra-articular steroids). In both cases, pain relief and function improvement was significant, but at 6 months patients treated with O_3 alone had a worse outcome compared with patients who received O_3 + corticosteroids.

Three studies compared O_3 with HA injections. Although all these studies showed overall comparable outcomes between these 2 treatments at short-term evaluation, some differences were reported. Duymus et al.[20] showed superior results for HA at the 3-month evaluation, whereas Invernizzi et al.[17] showed that O_3 provided a lower reduction of pain compared with HA at 1 month. In the aforementioned study by Duymus et al.,[20] aside from HA, O_3 also was compared with platelet-rich plasma (PRP) therapy, and the authors documented better outcomes in terms of VAS and WOMAC for the PRP group at 3 months.

Discussion

The main finding of the present systematic review is the overall poor quality of the available evidence concerning OOT in the treatment of KOA. Despite only including RCTs, critical assessment revealed relevant bias in all 11 studies considered, which do not allow to clearly understand how O_3 therapy compares with "standard" approaches currently adopted for KOA. O_3 was tested against pharmacologic agents and other common injections such as corticosteroids, HA, and PRP: unfortunately, the low number of trials found, with different clinical scores adopted, did not allow the authors to perform a meta-analysis of the results. Unexpectedly, all the RCTs analyzed in the present review are characterized by weak power analysis, in most cases lacking a clear statement concerning the primary outcomes and the numerical data used to calculate the sample size, which is therefore at high risk of being underpowered with obvious consequences on the significance of results. Recent high-quality RCTs focusing on injective treatments for OA have included almost 100 patients per treatment arm, exceeding by far the average number of patients treated in the present RCTs.[23,24]

Furthermore, there is an overall modest adherence to the Consolidated Standards of Reporting Trials guidelines for reporting methods and results in RCTs, thus generating a series of consecutive biases responsible for the very modest judgment of the trials according to the Agency for Healthcare Research and Quality standard: none of them in fact could be evaluated as a "good-quality" RCT. This finding reflects the current tendency to prefer quantity over quality of publications, following some sort of "publish or perish" approach.[25] In fact, the label "randomized controlled trials" is no longer sufficient to accurately identify high-quality studies, and it may be misleading for readers and at times also for reviewers. The differences between RCTs can be huge, in terms of blinding (from no blinding to single or double blinding), sample sizes, inclusion bias, outcome measures selection, and so on. In the case of OOT for KOA, the 11 RCTs published until now are not able to shed any light on the therapy's real potential compared with other treatments. This finding suggests that the current clinical application of OOT is not backed up by robust scientific data: this is not an "uncommon" situation in orthopaedics and, for example, a similar scenario characterized, in the recent past, the "routinary" clinical application of many biologic products for cartilage repair.[26,27]

Well-designed, multicentric RCTs are still necessary to elucidate many unanswered questions. To this regard, one of the main issues to consider is the large variability of therapeutic protocols carried out in the trials and specifically the amount and concentration of oxygen–ozone mixture, the administration frequency, and the injection technique: all these variables make study comparison very complex. O_3 concentration administered in a single injection ranged between 2 and 40 μg/mL and between 5 and 50 mL in terms of volume. According to some authors,[28] O_3 concentration within the O_2–O_3 mixture is the most crucial factor for determining the biologic effects of the treatment. In 4 cases, the protocol consisted in a weekly administration for 3 to 8 weeks, in 3 cases the injections were repeated 2 or 3 times in a week for up to 6 weeks, whereas 3 studies provided a single administration, thus leading to significant difference in the total quantities of O_3 administered among the different trials. At present, there is no unanimous protocol for O_3 treatment in KOA.[29] Guidelines from the International Scientific Committee of Ozone Therapy (ISCO3)[30] have been released, which suggest the avoidance of high volumes of OOT, but the lack of international standardization fosters further investigation to identify the best applicative modalities.

Nevertheless, despite the aforementioned relevant methodologic limitations, some clinical consideration can be drawn from the analysis of the literature, which underlines overall promising results of OOT in reducing pain and improving the functional status of patients affected by KOA. In particular, it proved to be safe, with an almost null adverse event rate: O_3 is bacteriostatic, fungicidal, and virucidal, therefore with minimal infection risk.[16] The average follow-up, similarly to other injective trials, was performed in the short-to-middle term, with just 1 study[20] reporting data up to 1 year of follow-up. In light of this, our primary aim was to understand the potential of OOT in terms of pain relief at short-term evaluation, being the median follow-up of all the included studies 3 months (range 1-6), which therefore was considered the reference time frame for the present review. Pain is also the main factor determining joint functional recovery and therefore it was considered as our primary outcome for data interpretation. It seemed that O_3 could provide the best results in terms of symptomatic relief within the first 3 months, with a gradual waning of the benefits over time, as already documented for any other injective treatment.[23] Based on this, after the first treatment, many physicians usually repeat injections to ensure a longer-lasting effect.[2] In regards to the K-L score, O_3 provided better results in grade I and II rather than in grade III. However, several authors support that severe OA is not a contraindication for O_3 administration: patients' improvement is significant and comparable with lower stages of OA, but effect duration is significantly shorter.[2]

When it comes to compare the efficacy of O_3 with other treatments, the aforementioned poor quality of

RCTs has a deep (negative) impact on the reliability of the findings. Four studies compared O_3 with corticosteroids,[14,15,18,21] and a cautious evaluation of data would suggest an overall superior efficacy of OOT over corticosteroids, especially in a middle-term time frame. O_3 was able to reduce some inflammatory cytokines' (interleukin-1b and tumor necrosis factor-alpha) serum levels, and it possibly displayed more stable anti-inflammatory effects compared with the steroid.[15] It is also interesting that the concurrent use of both O_3 and steroid injections could relieve symptoms much more efficiently than O_3 alone.[18] The major effects related to the combined use of O_3 and a nonsteroidal anti-inflammatory drug or steroid could be related to the action on different and independent metabolic pathways. The indirect anti-inflammatory action determined by the O_3 through the activation of antioxidant systems would be added to the direct anti-inflammatory and immunosuppressive action, exerted by steroid therapy or nonsteroidal anti-inflammatory drugs.

Three studies compared O_3 with HA intra-articular injections.[17,19,20] Both treatments proved to be effective in the management of pain and other OA-related symptoms; however, results were conflicting regarding the duration and impact of either approach.[19] It seemed that OOT was responsible for faster pain reduction, whereas HA showed longer lasting efficacy. Nevertheless, the combination of both treatments might lead to a significantly better outcome compared with HA and O_3 given separately.[22]

One single study investigated O_2–O_3 against PRP,[20] the latter showing better results especially in the long term. Some authors advocate that this result could be due to the high doses of O_3 administered in this study.[14] Regardless, given the small number of patients included in the trial, the superiority of PRP needs to be still clearly demonstrated.

The comparison with selective cyclooxygenase-2 inhibitors[22] showed a greater efficacy of OOT at short term but comparable results up to 6 months' evaluation. Lastly, radiofrequency or prolotherapy, both evaluated in a single study, showed similar results in term of pain relief and functional improvement with respect to O_3.[8,16] On the basis of the available data, OOT is able to provide significant pain reduction at least at short-term follow-up with overall decrease of the effect at longer evaluation. In conclusion, the RCTs currently available compared OOT with many different approaches for the treatment of KOA, with overall conflicting findings and no clear evidence of OOT superiority to any of the comparators. Nevertheless, the present authors believe that the use of OOT for KOA should not be discouraged, given the favorable safety profile and the encouraging results but, at present, it cannot be preferred or recommended over other approaches. The lack of well-designed RCTs is the main culprit for the present doubts, and this further testifies the fact that low-quality evidence is detrimental both for the scientific community and for patients.

The main unanswered question concerns the identification of the best therapeutic protocol of OOT in the treatment of KOA. Then, properly powered and well-conducted RCTs could elucidate the potential of OOT compared with other established treatment options.

Limitations

The present manuscript presents some limitations. First, a meta-analysis of data was not performed: the only possible attempt in this regard could have been to compare OOT with HA, but the low number of trials and the poor homogeneity of data would have resulted in an unreliable evaluation. Furthermore, despite being a systematic review of RCTs, the poor quality of the trials prevents the authors from defining clear indications on the comparative efficacy of OOT versus other approaches, thus preventing clinicians to obtain "practical" indications to be adopted in their practice.

Conclusions

The analysis of the available RCTs on OOT in the treatment of KOA revealed poor methodologic quality, with most studies flawed by relevant bias, thus severely limiting the possibility of drawing conclusions on the efficacy of OOT compared with other treatments. On the basis of the data available, OOT has, however, proven to be a safe approach with encouraging effects in pain control and functional recovery in the short-middle term.

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