World
Federation of
Ozone
Therapy

Headquarters:
Via de Pratello, 8
Bologne (Italy)
WEB: www.wfoot.org
Email: info@wfoot.org

Guangzhou, June 12th, 2017

Some medical ozone associations are promoting the use of ozonated saline solution as a method classifiable as Ozone Therapy. This is documented also by the "Second edition of Madrid Declaration on Ozone Therapy" edited by ISCO3 (International Scientific Committee on Ozone Therapy) that included this technique in their training courses.
The World Federation of Ozone Therapy – WFOT, very worried about this fact, has made a deep study through its scientific advisory committee that concludes:

- Ozonated saline following the recommendation from the above related document, introduces in the body a very low amount of ozone dissolved in certain quantity of saline solution, compared with systemic indirect endovenous ozone therapy (SIEVOT), also known as major autohemotherapy. This small quantity doubtfully would induce any significant biological response, through the generation of ROS and LOPs. We must say that it is not an Ozone Therapy technique based on the ozone chemistry well documented by the German, Cuban and Italian schools, in which ozone is the only molecule that interacts with the body fluids.

- The ozonation of saline solution (0,9% NaCl) induces the generation of dangerous oxidized chlorine derivatives, not present in other techniques of medical ozone administration. Ozonated saline solutions showed to induce mutagenicity and toxicity in clinical reports. Ozonated saline solutions lacks of any kind of approved preclinical studies to support its safety, as ozone has, which were developed in Cuba and following the recommendations of the World Health Organization – WHO.

For these reasons, WFOT cannot admit ozonated saline solutions as part of ozone therapy meanwhile the biochemical reactions, biological effects and safety of this procedure have not been even minimally established.

Attached, you can find the details of the WFOT - Scientific Advisory Committee study.

Prof. Dr. He Xiao Feng
WFOT President

Prof. Dr. José Baeza-Noci
WFOT Past President

Headquarters:
Via de Pratello, 8
Bologne (Italy)
WEB: www.wfoot.org
Email: info@wfoot.org

STUDY ON THE SCIENTIFIC BASIS OF OZONIZED SALINE SOLUTION
WFOT Scientific Advisory Committee
Originally written by Manuel Gomez-Moraleda

Safety.

The first reference[1] on ozonated saline solution comes from Korolev in 1977. A detailed review in Pubmed or Embase did not find any reference on safety preclinical studies in spite of 40 years of use.

The theoretical safety of this method was tried to argue by Razumovskii et al. publication[2] that states that "the decomposition of ozone in the aqueous solution of NaCl is not accompanied by the formation of products different from the oxygen, and no noticeable amounts of hypochlorites and chlorates were observed in particular." Nevertheless, the promoters of this method warn not to use ozone concentrations higher than the very low limit that they recommend (3 µg/mL). Therefore, this seems to indicate their recognition that something different from ozone, oxygen and NaCl (sodium chlorate) is present in this method.

However, Levanov et al. tried to reproduce the experience[3] and found methodological mistakes in Razumowsky work, because hypochlorite cannot be properly determined by direct spectrophotometry, as it has been used, because there are severe interferences between O_3 and Cl^-. Moreover, the method used to determine chlorate, produced volatile HOCl (hypochlorous acid), not measured in the experiment. Levanov states that other authors[4] have used iodimetric method to asses HOCl in dilution process of ozone in aqueous NaCl solution and found a progressive amount of HOCl in time. Regarding chlorate, even at very low concentrations, as detected by Grguric, induce oxidative destruction of erythrocytes[5].

But a greater danger in ozonated saline does not only arise from NaCl but from bromide ions that are always present together with Na and Cl ions and are not regulated. Ozone can easily induce the formation of HOBr (hypobromous acid) or bromates, that are potentially carcinogenic[3]. We can find in the literature more works that support Levanov critics[6].

Bocci[7,8] clearly tested that the speed of ozone absorption into saline is greater and its disappearance faster, due to the interaction with NaCl molecules. It is clear from this that ozone really reacts with NaCl. (Fig.1).

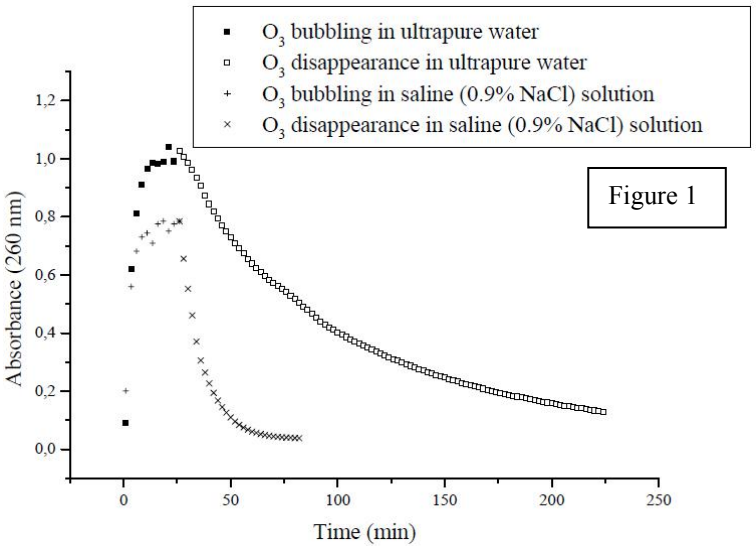

Figure 1

In these documents, Bocci states that even at ranges of 2-3 µg/mL of medical ozone, a proportional amount of ClO- (hypochlororate) is generated, inducing more oxidative stress and risk of DNA damage.

Headquarters:
Via de Pratello, 8
Bologne (Italy)
WEB: www.wfoot.org
Email: info@wfoot.org

Regarding this fact, in 1999, Fokinski et al.[9] detected an increase in oxidative damage markers in leucocytes of patients with arteriopathy treated with ozonated saline. This marker (8-oxodeoxyguanosine) is related with ageing and cancer[10], so the theoretical risk pointed out by Bocci confirmed to be real. One of the most important dangers is that these authors refer to this method as “treatment with Ozone” when it is actually treatment with Ozone mixed with several byproducts that will also interact with the body fluids. This risk has not been reported from SIEVOT.

Other authors[11] have confirmed the “hyper coagulation syndrome” as a possible complication of ozonated saline when ozone concentration is over 3 µg/mL. This may be reason not to increase the ozone concentration for the ozonation process.

Efficacy.

Using the methods referred in the Madrid Declaration[1] to generate ozonated saline and with the comments of the first chapter on top ozone concentration, the amount of ozone administered is 10 times lower than in a standard SIEVOT procedure.

According to the formulae used in this document, in a 80 kg patient, using 200 mL to 400 mL of saline solution (NaCl 0,9%) and a maximum solubility of 25% at 20ºC, they recommend:

- For low dose:

80 kg x 20 µg/kg = 1600µg (1,6 µg/mL) 1,6 x 0,25 = 0,4 µg/mL. ozone concentration in saline solution.
TOTAL DOSE = 0,4 x 200 mL = 80 µg per session or 0,4 x 400 mL = 160 µg per session.

- For medium dose:

80 kg x 40 µg/kg = 3200 µg (3,2 µg/mL) 3,2 x 0,25 = 0,8 µg/mL ozone concentration in saline solution.
TOTAL DOSE = 0,8 x 200 mL = 160 µg per session or 0,8 x 400 mL = 320 µg per session.

- For high dose:

80 kg x 100 µg/kg = 8000 µg (8,0 µg/mL) 8,0 x 0,25 = 2 µg/mL ozone concentration in saline solution.
TOTAL DOSE = 2,0 x 200 mL = 400 µg per session or 2,0 x 400 mL = 800 µg per session.

Advises on the usefulness of each dosage claim that Low dose (0,4 µg/mL) is used to stimulate the immune system, cardiovascular diseases and obstetrics. Medium dose (0,8 µg/mL) is recommended for endotoxemia and chronic inflammation diseases. High dose (2 µg/mL) is used for infections, skin injuries and burns. These advises are not supported by studies or publications in peer reviewed international journals.

Recently, a new paper from China[12] has confirmed the production of chlorate when ozonating saline solution with 100 mL of medical ozone at 20, 40 and 60 µg/mL concentration. The amount of chlorate is directly proportional to the dose; we admit that the minimum dose used in the work is 2.5 times greater than the maximum used

Headquarters:
Via de Pratello, 8
Bologne (Italy)
WEB: www.wfoot.org
Email: info@wfoot.org

recommended by the Nyzhny Novgorod school and more test should be done, but even 2.5 times lower chlorate amount could be potentially dangerous, and no endovenous cholorate preclinical tests have been done in the world (only for the oral way of administration[5]).

In a recent (2014) presentation of Borrelli[13] during EUROCOOP meeting, she compared 1000 µg total dose per session versus initial 3750 µg dose and subsequent 5000 µg dose in COPD and artheropathy, proving that lower dose had no biological neither therapeutic effect.

Bocci compares both method in the following table[8] (table 1).

Table 1 Conceptual and practical differences between major oxygen-ozone therapy and ozonated physiological saline

	M-OOT	OS
Volume	150–200 ml[a]	~250–500 ml[b]
Reaction sites	*Ex vivo*, in the glass container	*In vivo*
Biochemical effects	Reactive oxygen species, *ex vivo* alkenals, *ex vivo* and *in vivo*	*In vivo*
Average ozone dose	4–16 mg	0.75–1.5 mg

M-OOT, major oxygen-ozone therapy; OS, ozonated physiological saline.
[a]Usual ex-vivo blood volumes with Na-citrate or heparin in an ozone-resistant glass bottle. [b]The volume variability depends upon gender, flow rate and venous blood flow (the higher the flow, the lower the biochemical effect).

Reading both papers one can hardly expect any effectiveness of ozonated saline at the proposed dosage by the Russian school according to the mechanisms we know. Maybe other biochemical mechanisms could be involved in the related benefits, but nothing to do with the well described ozone generation of ROS and LOPs and their biological effects referred in other WFOT documents[14].

Headquarters:
Via de Pratello, 8
Bologne (Italy)
WEB: www.wfoot.org
Email: info@wfoot.org

References.

1 Madrid Declaration on Ozone Therapy. Second edition. ISCO3. Madrid, 2015.

2 Razumovskii, S.D., Konstantinova, M.L., Grinevich, T.V. et al. Mechanism and kinetics of the reaction of ozone with sodium chloride in aqueous solutions.Kinet Catal. 2010;51:492.

3 Levanov A.V, Antipenko E.E, Lunin V. Primary Stage of the Reaction between Ozone and Chloride Ions in Aqueous Solution: Can Chloride Ion Oxidation by Ozone Proceed via Electron Transfer Mechanism? Russian Journal of Physical Chemistry A. 2012;86(4):584-589.

4 Grguric G, Trefry JH, Keaffaber JJ. Ozonation Products of Bromine and Chlorine in Seawater Aquaria. Water Res. 1994;28:1087.

5 Chlorite and Chlorate in Drinking_Water. Background, Document for Development of WHO Guidelines for Drinking_Water Quality, WHO/SDE/WSH/05.08/86. (World Health Organization, Geneva, 2005)

6 Kang N, Jackson WA, Dasgupta PK, Anderson TA. Perchlorate production by ozone oxidation of chloride in aqueous and dry systems..Sci Total Environ. 2008 Nov 1;405(1-3):301-9. doi: 10.1016/j.scitotenv.2008.07.010. Epub 2008 Aug 21.

7 Bocci V, Zanardi I, Travagli V: Answer on letter to professor E.I. Nazarov (from Velio Bocci). http://ozonetherapy.org/04.02.2010/answer-bocci-nazarov/ accessed on 05/24/2017

8 Bocci V, Zanardi I, Borrelli E, Travagli V. Reliable and effective oxygen-ozone therapy at a crossroads with ozonated saline infusion and ozone rectal insufflation. J Pharm Pharmacol. 2012; 64(4):482-9. doi: 10.1111/j.2042-7158.2011.01427.x.

9 Foksinski M, Bialkowski K et al. Evaluation of 8-oxodeoxyguanosine, typical oxidative DNA damage, in lymphocytes of ozone-treated arteriosclerotic patients. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 1999;438(1):23-27.

10 Valavanidis A, Vlachogianni T, Fiotakis C. 8-hydroxy-2' -deoxyguanosine (8-OHdG): A critical biomarker of oxidative stress and carcinogenesis. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev. 2009 Apr;27(2):120-39. doi: 10.1080/10590500902885684.

11 Ikonomidis S, Tsaousis P, Fyntanis A, Iliakis EM: New data regarding the use of oxidative stress (ozone therapy) in the former Soviet Union Countries. Riv It Ossigeno-Ozonoterapia. 2005;4:40-43.

12 Ma L, Wen S, Yuan J, et al. Detection of chlorite, chlorate and perchlorate in ozonated saline. Exp Ther Med. 2020;20(3):2569-2576. doi:10.3892/etm.2020.9005.

13 Borrelli E. Reduction of oxidative stress index after major ozonated autohemotherapy: is the ozone concentration important? EUROCOOP, Zurich; 2014.

14 WFOT review on evidence based ozonetherapy. WFOT Scientific Advisory Committee. WFOT. 2015. http://www.wfoot.org/wp-content/uploads/2016/01/WFOT-OZONE-2015-ENG.pdf webcite accessed on 05/24/2017