# Medical Ozone Applications: Integrative Oncology

Brenden Cochran, ND, Virginia Osborne ND and Paul Anderson, ND

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#### You may smell Ozone after

a lightening storm



#### What is Ozone?

- VERY STRONG OXIDANT!
- Natural gas (O3). 3 atoms of Oxygen
- Powerful sterilizer in vitro:

bacteria, viruses, fungus and odors

- 3<sup>rd</sup> most potent oxidant after fluorine and persulfate
- O3 is very reactive (oxidation)
- A regenerative therapy
- Great Preconditioning agent

Ozone

03

# Only USE MEDICAL Ozone Generator





#### Ozone Generators

- Very reactive! Strong oxidant! Thus it will react with materials in the ozone machine including metals and plastics.
- We recommend an ozone generator with Quartz Glass and silicon tubes.
  - A lot of generators will have components in which ozone contacts metals, plastics, rubber, ceramics.
  - Ozone reacts with these materials and releases toxic byproducts into the ozone mixture.
- Want a machine with ozone destruct area (carbon). Otherwise ozone gets into the machine or your room slowly over time breaks down components.

#### Measuring Medical Ozone

1 Gamma = 1 mg/L = 1 mcg/ml

6γ vs. 20γ



#### 1 mcg/ml is the standard when describing dose

## Medical Ozone

Measuring Ozone

- 1 Gamma = 1 mg/L = 1 mcg/ml
- Need a pediatric regulator for these flow rates



FLOW	FLOW	07	OZONE CONCENTRATION SETTING ON GENERATOR									
LPM	cc/min	-		2	3	4	5	6	7	8	9	10
1/32	31	3	86	5	76	80	83	91	94	98	105	120
1/16	62	2	04	2	55	69	82	86	92	96	98	100
1/8	125	1	2 2	26	36	48	61	72	84	88	92	96
1⁄4	250	e	14	1	20	28	39	49	59	64	70	78
1/2	500	Э	5 7		11	15	22	27	36	40	44	50

## Dosing

- Low dose = 1 mcg/kg
  - Restoration/Regeneration
  - Support anti-oxidant pathways
- Medium dose = 2 mcg/kg
  - Neurological and cardiovascular
- High dose = 5 mcg/kg
  - Viral/Bacterial/Fungus

Madrid Declaration on Ozone Therapy 2<sup>nd</sup> ed. International Scientific Committee of Ozone Therapy

#### **Ozone Settings**

- Intra-articular/Paravertebral: 5 20 mcg/ml
- Subcutaneous: 5 10 mcg/ml
- Bags: 20 80 mcg/ml
- IV Major Autohemotherapy: 10 40 mcg/ml
- Minor Autohemotherapy: 5 40 mcg/ml
- Rectal ozone: 10 35 mcg/ml
- Ozonated waters/saline/dextrose: 70 80 mcg/ml
- Ozonated oils: 40 mcg/ml

Madrid Declaration on Ozone Therapy 2<sup>nd</sup> ed. International Scientific Committee of Ozone Therapy

# Pharmacology

#### Pharmacology

- Potent regulator of the immune system
- Stimulate increase oxygenation of tissues
- Activation of Platelets
- Improves circulation
- Other cellular impacts



Bocci, V. Biological and Clinical effects of ozone. Has ozone therapy a future in medicine? British Journal of Biomedical Science 1999, 36; 270-279



#### Immune

- Formation of ROS in the plasma
  - A rapid process which causes a 5-25% reduction in antioxidant capacity for 15-20 minutes.
    - Due to efficient recycling of oxidized compounds
  - Shifts and increase in H202 extracellular to increase gradient of H202 to move intracellular
  - Reacts with PUFA to form LOP(lipid oxidation products).
    - LOPs have a short half-life but can signal bone marrow, spleen, liver and other immune system with peroxidative stress.
    - This can up-regulate antioxidant enzymes (oxidative stress adaptation)

Bocci, V. Ozone. A New Drug 2<sup>nd</sup> Ed. 2011. Department of Physiology University of Siena

### Ozone (oxidant) upregulating antioxidants Literature

- Bocci V. Does ozone therapy normalize the cellular redox balance? Med Hypotheses 1996;46:150–154.
- Sharma YK, Davis KR. The effects of ozone on antioxidant responses in plants. Free Radic Biol Med 1997;23:480–488.
- Leon OS, Menendez S, Merino N, Castillo R, Sam S, Perez, et al. Ozone oxidative preconditioning:a protection against cellular damage by free radicals. Med Inflamm 1998;7:289–294.
- Barber E, Menendez S, Leon OS, Barber MO, Merino N, Calunga JL, et al. Prevention of renal injury after induction of ozone tolerance in rats submitted to warm ischaemia. Mediat Inflamm 1999;8:37–41.
- Larini A, Bianchi L, Bocci V. The ozone tolerance: I. Enhancement of antioxidant enzymes is ozone dose-dependent in Jurkat cells. Free Radic Res 2003;37:1163–1168.

#### Immune

- Inducing leukocyte activity
  - Increased production of cytokines such as interferon beta, gamma
    - Several interleukins (IL-1, IL-2, IL-8, TGF-B)

#### Immune

- Ozone is a disinfectant in vitro, **<u>NOT</u>** in vivo!
  - Cannot sterilize blood
- Will not inactivate bacteria, viruses, and fungi due to the pathogens powerful antioxidant systems.
- Thus Ozone is activating Neutrophils and stimulating multiple cytokines.

#### Oxygenation

- More then just increase oxygenation
- Increase in (2,3 DPG)
  - Remember from physiology this shifts the hemoglobin dissociation curve to the right
  - Thus increasing oxygen delivery to the tissues
- Mild increase in LOP cause upregulation of antioxidant enzymes which will improve metabolic characteristics of erythrocytes.

#### Improved Circulation

- Endothelium contact with LOPs which causes a release of NO(nitric oxide)
- Nitric oxide has as very short half life (2 msec) however the remote impact is due to reactions with NO and GSH, cysteine, albumin and hemoglobin.
  - This forms S-Nitrosothiols and S-nitrosohemoglobin (half life up to 50 minutes)
  - Excessive ROS produces toxic peroxynitrite (O=NOO-) and hypochlorite anions (ClO-)

#### Other cell impacts

- LOPs can reach organs such as hypothalamus, endocrine glands, kidney's, liver, bone marrow, etc.
- Causing:
  - Neuro-endocrine response (euphoria)
  - Upregulation of antioxidant enzymes
  - Induction of heat shock proteins

#### LOPs and bone marrow

- Possible that the LOP acute oxidative stress in bone marrow would activate MP-9 (metalloproteinases).
- This releases staminal cells to possible previous injury such as trauma or ischemic damage.

Cakir, Ruhi. Generaly Aspects of Ozone. Intech. http://dx.doi.org/10.57772/57470

#### High Dose IVC vs. H202 vs. Medical Ozone

#### ASC – High Dose IV:

- IV → ASC High Dose "Prodrug for H2O2 production"
- Plasma
  - ASC + Fe or Cu  $\rightarrow$  H2O2
  - Some reduced by plasma catalase and GSH peroxidase
- ECF
  - ASC + Fe or Cu  $\rightarrow$  H2O2
- Cell
  - Cytokine release / Immune stimulation PLUS:
    - Normal cell: H2O2 reduced by catalase to H2O
    - Abnormal cell: H2O2
      → potential cell
      damage

#### <u>H2O2 IV:</u>

- IV → H2O2
- Plasma
  - H2O2 catalase/Mn → H2O+O2
    → Plasma cytokine stimulation:
    - IL-1, IL-6, IFNa, TNF, NO
    - ALL H2O2 is dismutated in the venous circulation in seconds
- ECF
  - No H2O2 left But Increased Cytokine cascade → Immune stimulation
  - No H2O2 delivered to the cells

#### Ozone IV:

- IV  $\rightarrow$  Ozone  $\rightarrow$  H2O2 & LOPs
- Plasma
  - H2O2 catalase/Mn → H2O+O2
    → Plasma cytokine stimulation:
    - IL-1,IL-2, IL-6, IL-8, IFNa, TNF, NO, TGF-B
    - ALL H2O2 is dismutated in the venous circulation in seconds
- ECF
  - No H2O2 left But Increased Cytokine cascade → Immune stimulation
  - No H2O2 delivered to the cells
  - Lipid mediator stimulation

#### Review:

- Ozone utilizes the redox systems as a pro-oxidant
- Main actions are through H202 and LOPs
  - Lots of cytokine activity
- Antioxidant(s) pathway can be upregulated
- Therapeutically must have enough oxidants to have an effect.
- Thus very low dose ozone won't do anything.
  - 1 treatment is not enough, need multiple treatments.

# Conditions and Literature

#### Conditions Ozone has been used in treating:

- Acute and chronic bacterial, viral, and fungi
- Ischemic disease
- Age-related macular degeneration
- Neurodegenerative Disease
- Auto-immune
- Cancer
- Metabolic syndrome
- Orthopedic
- Fibromyalgia
- Dermatological diseases
- Dentistry
- Cosmetology

#### Cancer:

- Will have immune supportive effects like we see with H202 and ROS
- LOPs will also have impact
- Would be best to combined with other therapies such as alternating with IVC, H202, and antioxidant IVs (antioxidants NEVER on the same day)
- Most patients with metastatic disease resistant to radiotherapy and chemotherapy report improvement in quality of life with twice weekly ozone major AHT.
  - Thought to be due to LOPs neuroendocrine response

Cakir, Ruhi. General Aspects of Ozone. Intech. http://dx.doi.org/10.57772/57470

#### Ozone preconditioning Cisplatin

Pre treatment in rats with intrarectal applications of ozone to prevent nephrotoxicity.

Experimental groups	Serum Cr levels (µM)	GSH (nmol/mg of protein)	TBARS (nmol/ mg of protein)	SOD (SOD units/ mg of protein)	CAT (k <sub>15</sub> /g of wet tissue)	GSH-Px (IU/mg of protein)
Non-treated control	$67.9 \pm 11.73^{*}$	$9.3 \pm 0.90^{*}$	$0.25 \pm 0.014^{*}$	$8.7 \pm 1.15^{*}$	$6.8 \pm 0.39 *$	$6.0 \pm 0.25^{*}$
Oxygen-treated control	$70.6 \pm 6.61^{*}$	$8.9 \pm 0.96^{*}$	$0.26 \pm 0.012^{*}$	$8.8 \pm 1.26^{*}$	$6.7 \pm 0.63*$	$6.1 \pm 0.14^{*}$
Ozone-treated control	$69.2 \pm 7.61^{*}$	$8.5 \pm 0.47^{*}$	$0.31 \pm 0.034^{*}$	$8.6 \pm 1.72^{*}$	$6.7 \pm 1.56*$	$6.1 \pm 0.14^{*}$
CDDP control	$280.6 \pm 45.05$	$5.4 \pm 0.76$	$0.63 \pm 0.12$	$6.2 \pm 0.9$	$4.3 \pm 0.74$	$5.3 \pm 0.69$
$O_2$ -CDDP control	$288.4 \pm 44.03$	$4.9 \pm 0.81$	$0.55 \pm 0.029$	$6.6 \pm 0.94$	$4.3 \pm 0.49$	$5.5 \pm 0.55$
$O_3$ (0.36 mg/kg)+CDDP	$294.9 \pm 47.70$	$6.6 \pm 1.34$	$0.54 \pm 0.077$	$6.8 \pm 1.23$	$5.2 \pm 0.79$	$5.9 \pm 0.98$
$O_3$ (0.72 mg/kg)+CDDP	$106.0 \pm 29.78^{*}$	$12.3 \pm 1.17^{*}$	$0.48 \pm 0.049$	$9.1 \pm 0.81^{*}$	$5.5 \pm 0.67*$	$10.5 \pm 1.03^{*}$
$O_3$ (1.1 mg/kg)+CDDP	$130.4 \pm 30.41^{*}$	$13.4 \pm 2.7^{*}$	$0.29 \pm 0.062^{*}$	$9.1 \pm 1.01^{*}$	$6.0 \pm 0.54*$	$7.9 \pm 1.32^{*}$
$O_3$ (1.8 mg/kg)+CDDP	$288.4 \pm 47.81$	$10.9 \pm 1.57^{*}$	$0.22 \pm 0.03^{*}$	$6.1 \pm 0.9$	$5.9 \pm 0.57*$	$5.8 \pm 0.89^{*}$
$O_3$ (2.5 mg/kg)+CDDP	$392.9 \pm 47.56$	$10.6 \pm 1.76^{*}$	$0.28 \pm 0.033^{*}$	$3.0 \pm 0.48^{*}$	$6.1 \pm 64*$	$5.7 \pm 0.62^{*}$

Borrego, Aluet, et al. Protection by ozone preconditioning is mediated by the antioxidant system in cisplatin-induced Nephrotoxicity in rats. Mediators of Inflammation. 13(1), 13-119 (Feb. 2004)

#### Tumor Hypoxia

- Tumor hypoxia causes decreased apoptotic potential in treatments. Which can lead to resistance to radiotherapy and chemotherapy.
- Tumor hypoxia increases angiogenesis

#### Ozone Therapy for Tumor Oxygenation: a Pilot Study

Bernardino Clavo<sup>1,5</sup>, Juan L. Pérez<sup>2,5</sup>, Laura López<sup>1,5</sup>, Gerardo Suárez<sup>1,5</sup>, Marta Lloret<sup>1,5</sup>, Victor Rodríguez<sup>3</sup>, David Macías<sup>2,5</sup>, Maite Santana<sup>1</sup>, María A. Hernández<sup>1,5</sup>, Roberto Martín-Oliva<sup>2</sup> and Francisco Robaina<sup>4,5</sup>

<sup>1</sup>Radiation Oncology and Research Unit, <sup>2</sup>Medical Physics, <sup>3</sup>La Paterna Medical Center, <sup>4</sup>Chronic Pain Unit, Dr Negrín Hospital and <sup>5</sup>Canary Islands Institute for Cancer Research (ICIC), Las Palmas (Canary Islands), Spain

# Preconditioning for radiation-induced lung injury

Brazilian Journal of Medical and Biological Research (2013) 46: 789-796, http://dx.doi.org/10.1590/1414-431X20132856 ISSN 1414-431X

#### Effect of ozone oxidative preconditioning in preventing early radiation-induced lung injury in rats

and increased the activity of superoxide dismutase, which might indicate protection of the lung from radiation-induced lung injury. Serum tumor necrosis factor alpha and interleukin-1 beta levels, which increased significantly following total body irradiation, were decreased with ozone oxidative preconditioning. Moreover, ozone oxidative preconditioning was able to ameliorate radiation-induced lung injury assessed by histopathological evaluation. In conclusion, ozone oxidative preconditioning, repeated low-dose intraperitoneal administration of ozone, did not exacerbate radiation-induced lung injury, and, on the contrary, it provided protection against radiation-induced lung damage.

Key words: Ozone evidative preconditioning: Irradiation: Lung injuny

# Radiation induced Rectal Bleeding in Prostate Cancer

Hindawi Publishing Corporation Evidence-Based Complementary and Alternative Medicine Volume 2015, Article ID 480369, 7 pages http://dx.doi.org/10.1155/2015/480369

#### Research Article

#### Ozone Therapy in the Management of Persistent Radiation-Induced Rectal Bleeding in Prostate Cancer Patients

Bernardino Clavo,<sup>1,2,3,4,5</sup> Norberto Santana-Rodriguez,<sup>3,4</sup> Pedro Llontop,<sup>3</sup> Dominga Gutierrez,<sup>1,4</sup> Daniel Ceballos,<sup>6</sup> Charlin Méndez,<sup>3</sup> Gloria Rovira,<sup>7</sup> Gerardo Suarez,<sup>1,4</sup> Dolores Rey-Baltar,<sup>1,4</sup> Laura Garcia-Cabrera,<sup>1</sup> Gregorio Martínez-Sánchez,<sup>8</sup> and Dolores Fiuza<sup>3</sup>

#### **Rectal Ozone Insufflation**

- Insufflation of 150-300 ml
- Most common and effective concentration 20 ug/ml
- Treatment was 3 times per week
- Topical ozonated oils showed no impact due to oil not being absorbed well in the mucosa.

In conclusion, our results show a significant, and clinically relevant, effect of ozone therapy in the management of radiation-induced rectal bleeding. Following ozone therapy, the requirements for blood transfusions were significantly decreased as was the need for endoscopic procedures. The grades of toxicity (according to the CTCAE scale) were also reduced. These effects were maintained over the course of protracted follow-up, and no adverse impact on survival was noted. We believe the local application of ozone therapy can be useful as adjuvant treatment in managing radiation proctitis and, as such, merits further evaluation in randomized clinical trials.

# Applications of Ozone:

### **Applications:**

- Ozone major autohemotherapy
- Ozone minor autohemotherapy
- Rectal or vaginal insufflation
- Ozone injections
- Ozonated oils and water
- Limb bagging

#### Ozone major autohemotherapy:

- 50 200 ml blood taken from patient mixed with ozone (10 40 mcg/ml)
- Some techniques use 1:1 (blood:ozone)
- Some techniques use 1:2 (blood:ozone)
- EXTREMELY important to use PVC and DHEP

Free plastics (hard plastic). It is preferred to use sterile GLASS. (REMEMBER OZONE is unstable and reacts easily!)

• Only plastics for short infusions <15-20 min.



#### Ozone major autohemotherapy:

- Large quantities >100 blood, need a separate line to draw blood.
- Anti-coagulant must be used:
  - 3.13-3.8% Citrate or Heparin
  - 10 ml citrate per 90-100 ml blood
  - 15-20 IU Heparin per ml blood
- Don't use the same line to infuse,

this increases the risk of clotting in the line!

• Always use a **blood admin set!** This catches,

blood clots! A regular line will always clot due to filter size.



## Ozone major autohemotherapy:

- Never run in rapidly!
- Always us anti-coagulant
- Use glass when possible especially large Volume
- DON'T use anti-oxidants on the same day
- Selecting anti-coagulants
  - Sodium Citrate: Can be a safer choice
    - Caution with Hypocalcemia.
  - Heparin: May cause dyscoagulation
    - Severe hemorrhage for patients on: antico agents, hepatic disease, low prothrombin.
    - Can induce thrombocytopenia and platelet micro-aggregation using ozone concentrations near 80 gamma.
    - Heparin increases growth factor release from platelets and cytokines from leukocytes



## Rectal Insufflation:

- 100-800 ml ozone gas (10-35 mcg/ml) or ozonated water
- Insufflations done either:
  - 20 days in row and 3 weeks off
  - 2 days per week
- Measured increase in oxygen and LOPs in portal vein, jugular vein 30-60 min. after use. Parameters returned to baseline in 24 hours.
- Can retain for 20-30 min if looking to increase oxygenation.
- Possible equivalent to Ozone AHT.
- No serious adverse reports
- Best use: Preconditioning, Limb ischemia, GI infections, hepatic issues



#### Ozonated Oils:

- •Made with coconut, sunflower, olive, etc. oils.
- •40 gamma ozone bubbled through diffuser
- •Oil should turn into a jelly
- Take 24-36 hours of continuous bubbling to make and must clean the diffuser as it thickens.
- Best to purchase unless you have a lot of time
- Best use: topical rashes, wounds, ulcers.

#### Ozonated Water:

- Clean filtered water 250 1000 ml
- ■80 gamma for 5 25 minutes bubbled
- 1/2 life is 10 hours at 20 degrees Celsius
- 1/2 life is 24 hours in refrigeration with stored in glass with lid
- Best used: drinking, enema, vaginal, skin wash, dental cleaning, surface cleaning.

#### NEVER INFUSE IV!!!!!!

### Ozone Saline: Problems?

Holds ozone longer, some research of effect in Russia but.....

#### Safe topically and orally

Theoretically may be issue for infusion.

Small amounts of iron

H202 + Fe2+ ⇔ ·OH + OH- + Fe3+

Na+ Cl- +  $\cdot$  OH  $\Leftrightarrow$  NaClO + H2O2  $\rightarrow$  NaCl +H2O +2O

Don't want OCI- (hypochlorous acid) formed.

• This is inflammatory on endothelium

This causes microcoagulation

 However???? A world expert in the chemistry of ozone published in 2010 the decomposition of ozone in aqueous media of NaCl is not accompanied by the formation of hypochlorites and chlorates.

Madrid Declaration on Ozone Therapy 2<sup>nd</sup> ed. International Scientific Committee of Ozone Therapy

#### Ozone Glucose/water:

Holds ozone longer and stable for infusion

- Safe for slow IV (20-30 minutes), stability of 3 days (but remember we don't do this because D5W is Single dose usage!
- Not advised for diabetics

#### But does it really work????

#### ORP (Oxidation Reduction Potential)

- •Measures the release or acceptance of electrons. (Oxidization)
- •Most tap water is between 200 600 mv.
- •ORP over 800 is Water Sterilization
- •So lets look at solutions.

## Ozone 80 mcg/ml bubbled for 2 minutes.



## D5W

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## 11 COLL COLLE 1+03 SWI Milwaukee mV ON



## 0.9% NS

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#### HDIVC + ART + Ozonated Saline

- •HDIVC + ART infusions.
- •Then Ozonated Saline treatment.
- Patients reporting no SE.
- Some reporting more euphoria/energy

# Safety of Ozone:

## Work your patient up!

- Medications?
- Hepatic disease?
- Labs needed for Ozone: CBC, CMP, G6PD (Recent)
- Follow up labs 1-4 weeks
- If very ill support them first with anti-oxidants

#### Contraindications

- G6PD deficiency
- Thrombocytopenia < 50,000
- Severe cardiovascular instability
- Hemochromatosis
- Active convulsions
- Acute massive hemorrhage
- Toxic hyperthyroidism
- Active infusions or treatments with iron or copper



- Too high concentration ozone
- Not proper anti-coagulation and equipment
- Direct IV ozone can be DANGEROUS, remember 10-60 cc gas causes pulmonary embolism
- Deaths associated with using improper ozone generators and DIV (remember 95% is O2)

#### Dangers

- Don't ever inhale ozone!
  - The bronchial-pulmonary system is very sensitive to ozone.
  - The anti-oxidant pathways are much weaker then other tissues in the eyes, nasal and bronchial pathways.



- Ozone pharmacological action is believe to be due to LOPs and H202.
- Medical ozone is safe and effective with proper use and materials.
- Not a great stand alone treatment for cancer
- Helpful as an adjunctive treatment