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Rectal Administration and its Application in Ozonetherapy

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Key words: rectal insufflations, ozone, ozone therapy, autohemotherapy, rectal way

SUMMARY - *The rectal administration of ozone is one of the oldest systemic and local forms of application. The biological effects of the Rectal Insufflations of Ozone (RIO_3) has been demonstrated extensively either experimentally or clinically. Furthermore, preclinical studies demonstrated its low toxicity. RIO_3 has been now extended to treat many diseases and is increasingly being used as a systemic therapeutic form. RIO_3 is already being viewed as an alternative to Mayor autohemotherapy (MAH). Using standardized clinical protocols a therapeutic success can be reached with RIO_3. Handling the advantage and disadvantage of RIO_3, not as alternative to MAH but used properly (e.g. pediatric, geriatric, when MAH cannot be performed because i.v. is difficult due to unfavorable vein conditions, etc.), this method is a valid route of O_3/O_2 administration.*

Introduction

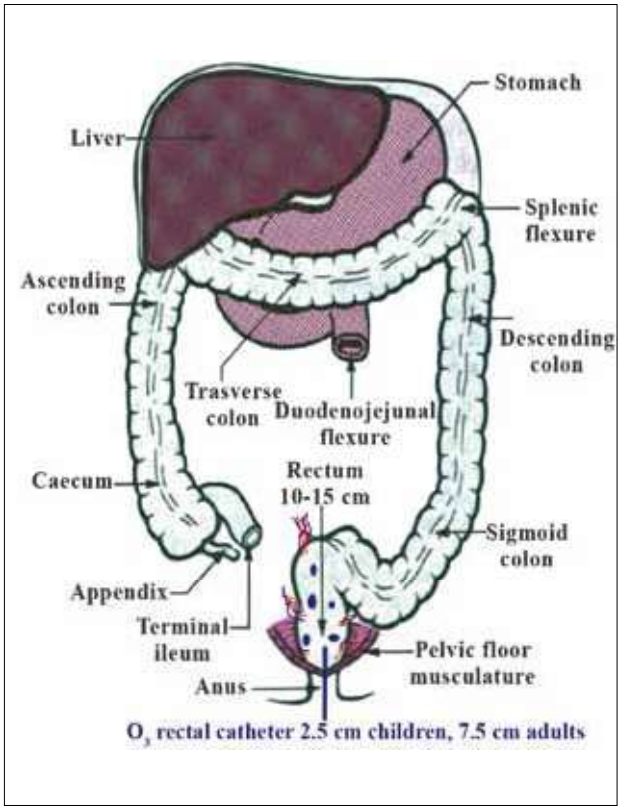
Rectal administration of drugs has been used since ancient times to produce local effects. In addition, the rectal route may be used for systemic administration of drugs [1]. The rectal administration of ozone is one of the oldest systemic and local forms of application [2-4]. Rectal insufflations of ozone (RIO_3) was first proposed by Aubourg (1936) for treating chronic colitis and fistulae [4]. Actually, the biological effect of RIO_3 has been demonstrated extensively either experimentally [5-7] or clinicaly [8-13]. Furthermore, preclinical studies demonstrated its low toxicity [6,14]. That's why the application of RIO_3 has been now extended to treat many diseases.

Based on animal investigations and a comprehensive proctological study, rectal insufflation with an O_3/O_2 gas mixture is increasingly being used as a systemic therapeutic form, and is already being viewed as an alternative to Mayor autohemotherapy (MAH). In addition, it is the method of choice in pediatrics [15].The main disadvantage of RIO_3 are connected with: 1) The variation in doses because of: possible flatulence, the presence of a more or less abundant luminal content and the neutralization of O_3/O_2 by fecal material produce erratic absorption. 2) Composition, viscosity, pH and surface tension of rectal fluids have great effects on drug bioavailability. 3) It's hypnotized that the O_3/O_2 concentration used is too high and during prolonged use may be mutagenic [16]. 4) Not well accepted because cultural patients' attitudes to rectal drug administration.

However, prelicinical and clinical studies demonstrated that using standardized clinical protocols a therapeutic success can be reached using RIO_3. Handling the advantage and disadvantage of RIO_3, not as alternative to MAH but used properly (e.g. pediatric, geriatric, when MAH cannot be performed because i.v. is difficult due to unfavorable vein conditions, etc.), this method is a valid route of O_3/O_2 administration. The aim of this manuscript was to review the preclinical and clinical paper whose support the use of RIO_3 in clinical practice and current clinical protocol. In addition some basic aspect concerning the anatomy and physiology of the colon were reviewed.

Consideration of the rectum and anus anatomy and physiology

The large bowel is a closed receptacle, 1-7 m long, with an ileocaecal valve at its cephalad end, which prevents reflux, and the dentate line of the anus at the caudad end [17]. While the transverse colon always has a mesentery, the ascending colon has a mesentery in only 12% of people and the descending colon has one in 22%. The sigmoid colon also has a mesentery and is sometimes unusual long (dolicolon) a feature which facilitates torsion or volvulus. The rectum, totally sheathed in longitudinal muscle fibres, is continuous with the anal canal, where the external sphincter of voluntary muscle provides an additional sheath. The levator ani sling muscle



← Figure 1 Anatomical aspect of the large bowel, and schematic representation of the rectal ozone application (adapted from Irving and Catchpole) [18].

Figure 2 A schematic figure showing the thicknesses of the → 2 mucus gel layers *in vivo* in different region of the rat gastrointestinal tract (adapted from Atuma et. al.) [36].

acutely angles (at 60°-105° in normal subjects) the rectoanal junctions forwards, its nerve supply running on its upper aspect and thereby being liable to damage by inordinate stretching of the muscle, for example, during childbirth (Figure 1) [18].

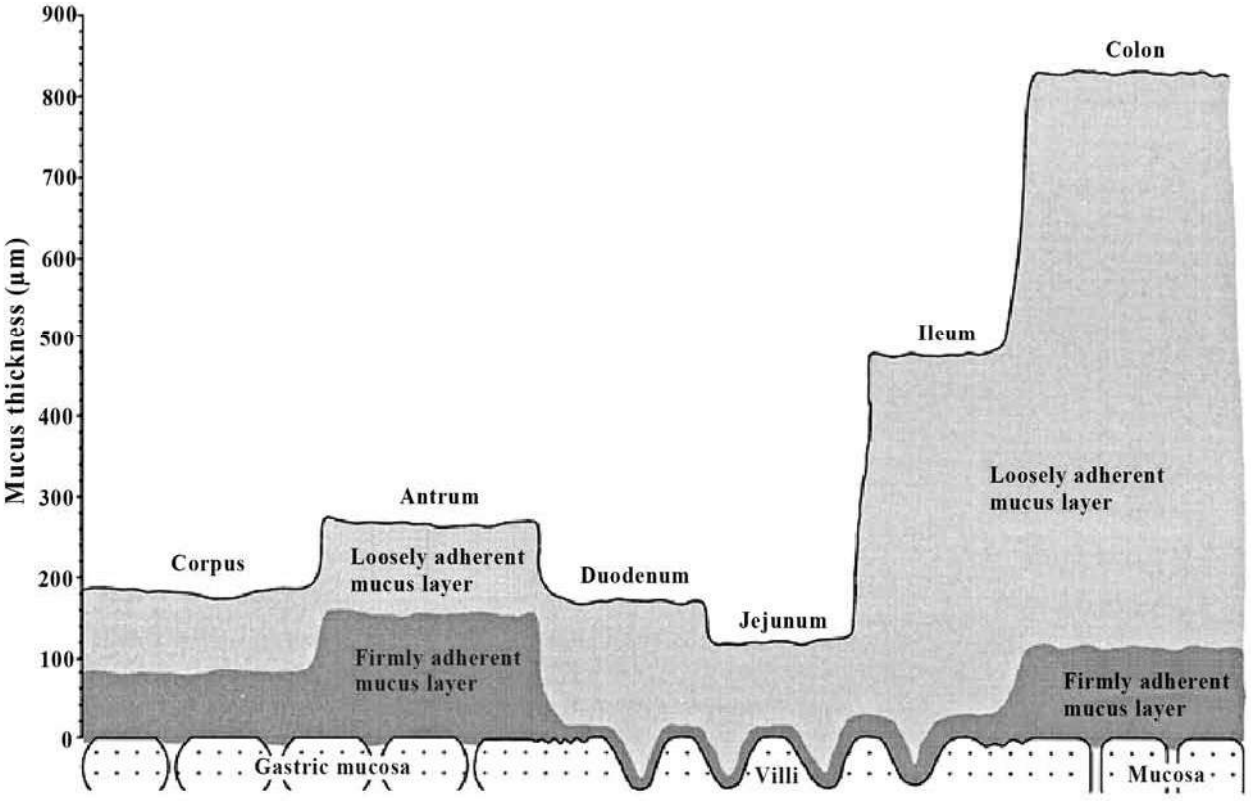
The colorectum is lined with columnar epithelium as far as the dentate line in the middle of the anal canal, where sensitive squamous epithelium in continuity with that of the perineum takes over. Submucous anal glands may extend deeply into the sphincter. The anal canal has a high pressure zone resulting from tonic contraction of the internal and external sphincters, which is responsible for continence. Voluntary contraction can, however, double this pressure (squeeze pressure). Anorectal sensation permits discrimination of solids from gas [18]. The pressure induced by rectal insufflation of O_3 during therapy also stimulates continence. Patients should be advised of this fact and will be invited to control this sensation at least for 5 min.

The rectum is normally empty, and when people awaken and eat breakfast, enhancing left colonic motility, faeces enter the rectum, and the person is called to stool. Sitting on the toilet helps to straighten out the anorectal angle and faeces enter the anal canal, to be passed if the passage is not voluntarily stopped. Further faeces from as far cephalad as the splenic flexure may be passed, the average daily volume being 150 mL. It is possible to delay expulsion: the rectum can accommodate passively adistension of up to 400 mL, maintaining a low rectal pressure, and faeces may even be propelled back into the sigmoid colon [18,19].

During RIO_3, once O_3 enter into the rectum the substrate of reaction will be feces, flatus and mucus. The main characteristics of those components are:

Mucus Secretions. The mucosa of the large intestine, like that of the small intestine, has many crypts of Lieberkühn; however, unlike the small intestine, there are no villi. The epithelial cells contain almost no enzymes. Instead, they consist mainly of mucous cells that secrete only mucus. The great preponderance of secretion in the large intestine is *mucus*. This mucus contains moderate amounts of bicarbonate ions secreted by a few non–mucus-secreting epithelial cells. The rate of secretion of mucus is regulated principally by direct, tactile stimulation of the epithelial cells lining the large intestine and by local nervous reflexes to the mucous cells in the crypts of Lieberkühn [18].

Mucus in the large intestine protects the intestinal wall against excoriation, but in addition, it provides an adherent medium for holding fecal matter together. Furthermore, it protects the intestinal wall from the great amount of bacterial activity that takes place inside the feces, and, finally, the



mucus plus the alkalinity of the secretion (pH of 8.0 caused by large amounts of sodium bicarbonate) provides a barrier to keep acids formed in the feces from attacking the intestinal wall [20].

The mucus of the small intestine has only one layer, whereas the large intestine has a two-layered mucus where the inner, attached layer has a protective function for the intestine, as it is impermeable to the luminal bacteria [20]. The thicknesses of the 2 mucus gel layers are particularly reinforces in the colon (Figure 2) [17].

Composition of feces and flatus. The feces normally are about three-fourths *water* and one-fourth *solid matter* that itself is composed of about 30% *dead bacteria*, 10% to 20% *fat*, 10% to 20% *inorganic matter*, 2% to 3% *protein*, and 30% *undigested roughage* from the food and dried constituents of digestive juices, such as bile pigment and sloughed epithelial cells. The brown color of feces is caused by *stercobilin* and *urobilin*, derivatives of bilirubin [18]. Short chain fatty acids (acetate, propionate, butyrate) are metabolic products of anaerobic bacterial fermentation of dietary fiber and resistant starch, are also present in the colon luminal content [21].

The odor is caused principally by products of bacterial action; these products vary from one person to another, depending on each person's colonic bacterial flora and on the type of food eaten. The actual odoriferous products include *indole*, *skatole*, *mercaptans*, and *hydrogen sulfide* [18,22]. Gases produced intraluminally (H_2, CO_2, and CH_4) comprised approximately 74% of flatus, and rapid CO_2 and H_2 productions were responsible for high passage rates. A positive correlation between flatus H_2 and CO_2 suggested that CO_2, like H_2, mainly was a bacterial product. Whereas methanogens and H_2S-producing bacteria usually are mutually exclusive in feces, CH_4 and H_2S did not negatively correlate, indicating coexistence of both organisms in the colon [23].

The dose of O_3 applied directly in the rectum during RIO_3, is evidently reduced in different proportions because it's reaction with the luminal content (flatus, feces and mucus). To by past this effect, the German School of ozonetherapy assume empirically a tree fold increment in the rectal dose compared to the same dose administered by the MAH. For example, for a dose of 1.5 mg (30 µg / mL / 50 mL) by autohemotherapy, the corresponding rectal dose will be 4.5 mg (22.5 µg / mL / 200 mL) [15].

Blood vessels from the lower part of the rectum connect with the inferior vena cava instead of merging into the portal vein. The rectal tissues are drained by the inferior, middle and superior haemorrhoidal veins, but only the superior vein connects with the hepatic-portal system (Figure

Table 1 Some preclinical studies using rectal administration of ozone.

Animal model of	*Animal Specie*	*Dose* mg/kg	*O_3 concentration* (µg) / [*volume* (mL)] / (No. Sessions)	*Results* (in brief)	*Reference*
Hepato cellular damage	Rats	1.0	50 [4.4-5] (15)	Hepato protection	León F, et al. 1998 [37].
Renal ischaemia	Rats	0.5	50 [2.5-2.6] (15)	Reduce renal damage	Barber E. et al. 1999 [38].
Hepatic ischemia-reperfusion	Rats	1.0	50 [4.4-5] (10)	Protective effect	Peralta C. et al. 1999 [39].
STZ-induced diabetes	Rats	1.1	50 [5-5.5] (10)	Reduces markers of oxidative and endothelial damage	Al-Dalain S.M. et al. 2001 [40].
Hepatocellular damage	Rats	1.0	50 [4.4-5] (15)	Prevent anaerobic glycolysis and oxidative stress induced by CCl_4.	Candelario-Jalil E. et al. 2001 [41].
Hepatic ischaemia-reperfusion	Rats	1.0	50 [5-5.5] (15)	Similar mechanisms of protection of ischaemic and ozone oxidative Preconditionings	Ajamieh H.H. et al. 2002 [42].
Hepatic ischaemia-reperfusion	Rats	1.0	50 [5-5.5] (15)	Hepato protection	Ajamieh H.H. et al. 2004 [43].
Acute nephrotoxicity	Rats	0.36 [a] 1.1 1.8	10 [9] (5) 30 [9] (5) 50 [9] (5)	Renal protection	González R. et al. 2004 [7].
Cisplatin-nephrotoxicity	Rats	0.36/0.72/ 1.1/1.8/ 2.5	20/30/50/70 [9](15)	Renal protection	Borrego A. et al. 2004 [44].
Hepatic ischaemia-reperfusion	Rats	1.0	50 [5-5.5] (15)	Protein synthesis is involved in the protective mechanisms	Ajamieh H.H. et al. 2005 [45].
STZ-induced diabetes	Rats	1.1	50 [5-5.5] (10)	Preserved b-cells functions and reduced hyperglycemia	Martínez-Sánchez et al. 2005 [46].
Chronic renal failure	Rats	0.5	50 [2.5-2.6] (15)	Renal protection	Calunga et al. 2005 [47].
Cisplatin-nephrotoxicity	Rats	1.1	50 [2.5-2.6] (15)	Renal protection	Borrego A. et al. 2006 [48].
Hepato cellular damage	Dogs	1.9-2.4	20 [97-121](15) [c]	Hepato protection	Li-Jie L. et al. 2007 [49].
Parkinson	Rats	0.7	25 [5] (20)	Neuro protection	Re L. et al. 2008 [50].
Red blood cell rheology	Rabbits	1.5-0.94 [b]	20 [150] (15/21/36)	Improvement	Artis et al. 2010 [5].
Redox status	Rabbits	11 34 79	600 [30] (90) 1400 [40] (90) 2600 [50] (90)	Improvement	Guanche et al. 2010 [6].
Arthritis	Rats	0.5/0.7 /1.0 [e]	40-60 [5-6] (15)	Improvement	Mawsouf N. et al. 2011 [51].
Renal ischaemia-reperfusion	Rats	0.5	ND [ND] (10) [d]	Protective effect in preserving renal function and morphology	Fernández Iglesias A, et al. 2011 [52].
Endotoxic shock	Mice	0.2 0.4	(5)	Inhibits TNF-alpha production	Zamora Z.B. et al. 2004 [25].

Legend: [a] Tree groups with different doses; [b] tree groups with the same dose but different follow-up times (tree times schedules); [c] one group with progressive dose increment; [d] post ischemia reperfusion treatment; [e] one treatment every other day for 30 days. *ND*, non-defined.

Table 2 Selection of some clinical studies using rectal administration of ozone.

Diseases	*No. Sample*	*Dose* mg	*O_3 concentration* (µg) / [*volume* (mL)] / (No. Sessions)	*Result*	*Reference*
AIDS diarrhea	5	2.7-30	ND (21-28)	Effective	Carpendale M.T. et al. 1993 [53].
Arteriosclerosis obliterans	18	ND	ND	Improvement	Romero Valdés A. 1993 [8].
Asthma	37	10	50 [200] (20)	Improvement	Hernández Rosales F.A. et al. 2005 [10].
Type 2 diabetes	52	10	50 [200] (20)	Improvement	Martínez-Sánchez et al. 2005 [9].
Hypertensive Pregnant Women	15	3-12	20-40 [150-300] (21)	Improve the umbilical flow indices and reduce antihypertensive therapy	Tanbouli T. et al. 2009 [54].
Coronary Artery Disease	40	10	50 [200] (20)	Improvement	Delgado-Roche et al. 2011 [55].
Retinitis Pigmentosa	56	8	40 [200] (20) [a]	Improvement and increasing their quality of life.	Copello M. and Menendez S. 2011 [24].
Cerebral disorders	43	ND	15-25 [15-120] (20) [b]	Improvement	Diaz E. et al. 2011 [56].
Pulmonary emphysema	20	6	30 [200] (20) [c]	Improvement	Calunga F. J.L. et al. 2011 [13].
Portal vein oxygenation in liver cirrhosis	15	12	40 [300] 12	Improve portal vein oxygenation	Zaky S. et al. 2011 [12].
Coronary artery disease	26	8	40 [200] (20)	Improved prothrombin time, without modify bleeding time	Martínez-Sánchez et al. 2012 [57].

Legend: [a] treated twice a year during 20 years. [b] treated every 3 months for one year, ozone volume depend of the age (1 year 15-20 mL; 1-3 years, 20-35 mL; 4-10 years, 40-75 mL; 11-15 years, 75-120 mL). [c] treated every 3 months for six months. *ND*, non-defined.

1). Medicaments absorbed in the lower part of the rectum are delivered directly into the systemic circulation, thus avoiding any first-pass metabolism [1].

Evidence of the effectiveness and toxicity of rectal insufflation of ozone

Most of the preclinical model used to study the pharmacological effects of ozone therapy used the rectal way because it's applicability in experimental conditions. Selected examples are shown in Table 1. Dose ranges from 0.2 to 79 mg/kg b.w. were used. All case referred a positive pharmacological effect without side effects. The O_3 concentration was in general between 10 µg – 50 µg, with exception of highest dose in one experiment [6]. Tissue protections by a mechanism mediated by the synthesis of proteins (essentially antioxidant enzymes) was the main pharmacological effect finding in animal models.

Clinical trials using RIO_3 demonstrate its therapeutic effectiveness in different pathological conditions (Table 2). In all case it was reported no side effects, even in one of the longer clinical trials (patients was followed by 20 years) 24 no side effects was founded. Preclinical or clinical studies that compare the effect of RIO_3 to other administration ways, found controversial results, for example:

In a shock septic model in mice, pretreatment with O_2/O_3 was administered intraperitoneally (i.p.) (0.2 mg/kg, 0.4 mg/kg and 1.2 mg/kg) or by rectal application (0.2 mg/kg and 0.4 mg/kg) once daily during five days before lipopolysaccaride (LPS) (0.1 mg/kg, intraperitoneal). One hour after LPS injection, a significant increase of TNF-alpha in mouse serum was observed. Statistically significant decreases in TNF-alpha levels after LPS injection were observed either with ozone i.p. applications at 0.2 mg/kg (78%), 0.4 mg/kg (98.5%) and 1.2 mg/kg (98.6%) mg/kg or by rectal application at 0.2 mg/kg (46.2%) and 0.4 mg/kg (97.4%) [25]. In this model i.p. and rectal way dose 0.4 mg/kg were bio-equivalent in reduction of TNF-alpha.

In a clinical study in asthma patients using MAH or RIO_3 the lung function and symptoms test were markedly improved. However, in all parameters

the best response was obtained in the order: MAH at 8 mg better than MAH at 4 mg better than RIO_3 at 10 mg [10].

In a clinical trial in non-diabetic patients with obliterant atherosclerosis, stadium II, (intermittent claudication) there was a significant improvement in comparison to the control group (conventional medical treatment). The improvement was independent of the administration routes (RIO_3, MAH, i.m.) [8].

Probably a correspondence between the dose used in MAH and RIO_3 will be reached using standard protocol during RIO_3, that minimised the reduction in the real O_3 dose as a result of it reaction of with the luminal content. We should take into consideration that the dose used by MAH is also subjected to modification subject by subject. This happen as a result of the different content of antioxidant levels in serum.

In most of the preclinical assays there were not observed adverse effect during rectal application of ozone. Not even in a study that use 2600 µg (dose 79 mg/kg) repeated in 90 sessions was found any damage associated to the treatment [6]. The *in vivo* genotoxic effect of O_3/O_2 was studied in leukocytes and exfoliated colorectal cells of rats using the Comet assay (single cell gel electrophoresis assay, SCGE). O_3 final dose 42 mg/kg b.w. (525 µg) was applied during 4 days by RIO_3 simulating human RIO_3. The genotoxic effect of O_3 was measured in exfoliated colorectal cells at 24, 48 and 72 h and in leukocytes at 0, 2, 6, 24, 48 and 72 h after the last exposure to O_3. As a result, a significant increase of the primary DNA damage was observed in exfoliated colorectal cells as well as in the peripheral blood leukocytes. The highest values of DNA damage were observed at 48 h and 24 h after the last exposure to O_3/O_2 mix in exfoliated colorectal cells and in leukocytes respectively. However, after 72 h of the last exposure a significant decrease of DNA damage was observed in both cell types, indicating an evident recovery of the DNA primary damage induced by the treatment [14].

There are several reactive intermediaries of O_3 that could cause primary DNA damage to leukocytes; some of them are H_2O_2, aldehydes and other inorganic and organic peroxides [26]. These reactive intermediaries have different diffusion rates according their liposolubility and molecular dimensions [27]. Stated that H_2O_2 is the reactive oxygen specie that more easily cross cell membranes. This ability could make H_2O_2 the most probable candidate of the observed early effect of O_3 in lymphocytes. Another group of O_3 intermediaries that could be related to this are long chain aldehydes, such as hexanal, heptanal and nonenal [28,29]. Their diffusion rates are slower than that of H_2O_2, precisely because they have to overcome the energetic barrier, imposed by their liposolubility, in order to leave membranes and diffuse into the cytosolic environment. Little is known about the diffusion properties of the other O_3 intermediaries (ozonides, lipoperoxides) in biological systems, but their reactivity and liposolubility might determine a diffusion rate slower [30]. The decrease of Comet lengths 48-72 h O_3 after treatment could be a result of the tissue recovery by cell death, cell turnover and DNA repair. It has been reported that the repair of single DNA strand breaks caused by oxidative damage, occurs in a few minutes, while repair of double DNA strand breaks may take up to 1 h [31].

When leukocytes and colorectal cells DNA damage is compared, it is observed that DNA damage is higher in leukocytes than in colorectal cells [14]. This unexpected result can be due to the fact that colorectal cells are directly exposed to O_3 and might, therefore, display higher levels of DNA damage. The most probable explanation is that colorectal mucous epithelium, particularly the goblet cells, produces mucin providing a defence mechanism against toxic bio-products of metabolism, pathogenic micro-organisms and xenobiotics [14,18].

RIO_3 is used for the treatment of colitis [32]. However, a study that examine the effect of ozonized water (20 µg/mL) enema on normal and inflamed rat colonic mucosa shown that O_3 therapy caused no macroscopic damage. Nevertheless, O_3 therapy induced microscopic colitis, which lasted for at least a week and was accompanied by increase in segmental weight, myeloperoxidase and nitric oxide activity, and prostaglandin E2 generation. In addition, O_3 therapy had no protective effect on inflamed mucosa. This study suggesting that ozone water therapy had a deleterious effect on normal colonic mucosa [33]. In contrast, a study in rabbits, using Ozone 2600 µg (dose 79 mg/kg) repeated in 90 sessions was found that O_3 did not cause adverse effects and did not show significant changes relative to tissue damages and they increased enzymes activities belonging to the first line antioxidant defenses. These results demonstrate that ozone/oxygen mixture administered by rectal insufflations is innocuous and it is able to increase the antioxidant defense of the organism. In addition, most of the long term clinical studied did not found any collateral effects after RIO_3 application [24].

Procedure for rectal insufflation of ozone

RIO_3 is a method of ozone therapy second only to MAH [15]. RIO_3 should be done following right steps in order to guaranty the maximal efficacy of the procedure.

Preparation

Before administering rectal medicine, the door to the room should be closed to assure patient privacy. The patient should be encouraged to empty his or her bladder and bowels before the procedure. After removing lower garments and underwear, the patient should be positioned in bed on his or her left side, with the top knee bent and pulled slightly upward, lifting the upper buttocks will enable visualization of his or her rectal opening. A waterproof pad should be placed under the patient's hips to protect the bedding, and a sheet should be draped over the patient to cover all of his or her body except the buttocks.

After placing a bedpan within quick access, the nurse should explain the procedure to the patient. This explanation should include the importance of breathing slowly through the mouth to enhance relaxation of the rectal sphincter and to avoid oppositional pressure. The patient should be made aware that there may be an urge to push the medicine out, but that he or she should try to hold it for at least 10 - 15 min after instillation, as most rectal medications need time to be absorbed.

The nurse should wash his or her hands and put on gloves. The foil wrap should be removed from the rectal catheter. External lotions, ointments or creams can be applied directly, using a gloved finger or a 4×4 gauze pad. Prior to administering the tip of the catheter, or applicator should be lubricated with a water-soluble lubricant. To insert a rectal catheter, the lubricated, tapered end of the catheter should be placed at the rectal opening and gently pushed into the rectum. The catheter should be pushed continually toward the umbilicus until the full length of the nurse's gloved index finger has been inserted into the rectal opening (i.e., about 3 inches, or 7.5 cm, for an adult patient). When inserting a rectal catheter into children, the catheter should be pushed about 1 inch (2.5 cm) beyond the rectal opening, or up to the first knuckle of the nurses's index finger. When inserting a rectal catheter into infants, the little finger should be inserted one-half inch (1.25 cm) beyond the rectal opening. The buttocks should be released and the finger removed.

Volume and Concentration

A good starting point for most first time users is 100 mL (assuming the concentration is between 10 – 20 µg/mL). Volume and concentration will be adjusted progressively depend of the redox status and the particular pathology of the patient. However, concentration superior to 40 µg/mL and volumes higher than 300 mL are not recommended [34]. The concentration usually chosen for therapeutic effect from rectal insufflation is between 10 – 30 µg/mL. This concentration may be higher (up to 60 µg/mL) for treating bleeding or acute colitis, bacterial, or parasitic infections.

The volume of gas used is extremely important as well. Performing rectal insufflation is somewhat like blowing up a balloon. Too much gas could cause damage to the intestinal tract. Furthermore, increasing, or decreasing the volume of gas used will change the overall dose of ozone. For example, if the concentration used then is 20 µg/mL, and volume of gas 100 mL the dose correspond to 2 mg of O_3. For detail about the recommended dose see the Madrid Declaration on ozone therapy [35].

If the concentration used causes irritation or discomfort consider lowering the concentration used or discontinuing treatment until irritation subsides. In most case a cycle of 15-20 RIO_3 was practice with a rest time of 3 months, or adjusted depends on the pathology [34].

In summary, RIO_3 is a valid therapeutic choice in ozone therapy. Pre-clinical and clinical studies demonstrated that using standardized clinical protocols a therapeutic success can be reached.

References

1 De Boer AG, De Leede LG, Breimer DD. Drug Absorption by Sublingual and Rectal Routes. British Journal of Anaesthesia. 1984; 56 (1): 69-82.
2 Payr E. Uber Ozonbehandlung in der Chirurgie. Munch Med Wochenschr. 1935; 82: 220-91.
3 Aubourg P. Ozon in der Chirurgie. Mem Acad Chir. 1940; 65: 1183-1192.
4 Aubourg P. Colibacillose aigue, colibacillose cronique: ameliorations cliniques notables par un traitement d'ozone. Bull Med Paris. 1936; 140: 644-654.
5 Seda Artis A, Aydogan S, Gokhan Sahin M. The effects of colorectally insufflated oxygen-ozone on red blood cell rheology in rabbits. Clin Hemorheol Microcirc. 45 (2-4): 329-336.
6 Guanche D, Zamora Z, Hernandez F, et al. Effect of ozone/oxygen mixture on systemic oxidative stress and organic damage. Toxicol Mech Methods. 2010; 20 (1): 25-30.
7 Gonzalez R, Borrego A, Zamora Z, et al. Reversion by ozone treatment of acute nephrotoxicity induced by cisplatin in rats. Mediators Inflamm. 2004; 13 (5-6): 307-312.
8 Romero Valdes A, Blanco Gonzalez R, Menendez Cepero S, et al. [Arteriosclerosis obliterans and ozone therapy. Its administration by different routes]. Angiologia. 1993; 45 (5): 177-179.
9 Martinez-Sanchez G, Al-Dalain SM, Menendez S, et al. Therapeutic efficacy of ozone in patients with diabetic foot. Eur J Pharmacol. 2005; 523 (1-3): 151-161.
10 Hernandez Rosales FA, Calunga Fernandez JL, Turrent Figueras J, et al. Ozone therapy effects on biomarkers and lung function in asthma. Arch Med Res. 2005; 36 (5): 549-554.
11 Zaky S, Kamel SE, Hassan MS, et al. Preliminary results of ozone therapy as a possible treatment for patients with chronic hepatitis C. J Altern Complement Med. 2011 Mar; 17 (3): 259-263.
12 Zaky S, Fouad E, Kotb HIM. The effect of rectal ozone on the portal vein oxygenation and pharmacokinetics of propranolol in liver cirrhosis (a preliminary human study). Br J Clin Pharmacol. 2011; 71 (3): 411-415.
13 Calunga J, Paz Y, Menéndez S, et al. [Rectal ozone therapy for patients with pulmonary emphysema]. Rev Med Chile. 2011; 139: 439-447.
14 Diaz-Llera S, González-Hernández Y, Mesa JEG, et al. Induction of DNA primary damage in peripheral blood leukocytes and exfoliated colorectal epithelial cells in rats treated with O_3/O_2 mix. International Journal of Ozone Therapy. 2009; 8: 217-221.
15 Viebahn-Hänsler R. The use of ozone in medicine. 5th ed. Germany: ODREI; 2007. 1-176 p.
16 Bocci V, Borrelli E, Zanardi I, et al. Oxygen-ozone therapy is at a cross-road. Revista Española de Ozonoterapia. 2011; 1 (1): 74-86.
17 Johansson MEV. The MUC2 mucin. A network in the intestinal protective mucus. Gothenburg: Gothenburg, 2009. 58 p.
18 Irving MH, Catchpole B. Anatomy and Physiology of the Colon, Rectum, and Anus. BMJ. 1992; 304 (25): 1106-1108.
19 Stabile G, Minervini S, Basoli A, et al. [Anorectal functional study. The state of the art]. Minerva Chir. 1994; 49 (12): 1187-1193.
20 Johansson ME, Ambort D, Pelaseyed T, et al. Composition and functional role of the mucus layers in the intestine. Cell Mol Life Sci. 2011; 68 (22): 3635-3641.
21 Andoh A, Tsujikawa T, Fujiyama Y. Role of dietary fiber and short-chain fatty acids in the colon. Curr Pharm Des. 2003; 9 (4): 347-358.
22 Vysotskii VG, Vlasova TF, Kochetkova AN, et al. [Composition of urine and feces in healthy subjects]. Vopr Pitan. 1974; (6): 35-38.
23 Suarez F, Furne J, Springfield J, et al. Insights into human colonic physiology obtained from the study of flatus composition. Am J Physiol. 1997; 272 (5 Pt 1): G1028-G1033.
24 Copello M, Menéndez S. Retinitis Pigmentosa patients treated with ozone therapy during 20 years. Cuban experiences. Revista Española de Ozonoterapia. 2011; 1 (1): 13-22.
25 Zamora ZB, Borrego A, Lopez OY, et al. Inhibition of tumor necrosis factor-alpha release during endotoxic shock by ozone oxidative preconditioning in mice. Arzneimittelforschung. 2004; 54 (12): 906-909.
26 Marnett LJ. Lipid peroxidation-DNA damage by malondialdehyde. Mutat Res. 1999; 424 (1-2): 83-95.
27 Halliwell B GJMC. Free Radicals in Biology and Medicine. 2nd ed. Oxford: Clarendon Press; 1989.
28 Pryor WA, Bermudez E, Cueto R, et al. Detection of aldehydes in bronchoalveolar lavage of rats exposed to ozone. Fundam Appl Toxicol. 1996; 34 (1): 148-156.
29 Hamilton RF, Jr., Li L, Eschenbacher WL, et al. Potential involvement of 4-hydroxynonenal in the response of human lung cells to ozone. Am J Physiol. 1998 Jan; 274 (1 Pt 1): L8-16.
30 Pryor WA. How far does ozone penetrate into the pulmonary air/tissue boundary before it reacts? Free Radic Biol Med. 1992; 12 (1): 83-8.
31 Azevedo F, Marques F, Fokt H, et al. Measuring oxidative DNA damage and DNA repair using the yeast comet assay. Yeast. 2011; 28 (1): 55-61.
32 Knoch HG, Klug W. [Ozone-oxygen therapy in proctology]. Ter Arkh. 1990; 62 (2): 93-98.
33 Eliakim R, Karmeli F, Rachmilewitz D, et al. Ozone enema: a model of microscopic colitis in rats. Dig Dis Sci. 2001; 46 (11): 2515-2520.
34 Schwartz A, Martinez-Sanchez G, Re L. Guia para el uso médico del ozono. Fundamentos terapéuticos e indicaciones. Madrid; 2011.
35 AEPROMO. MADRID DECLARATION ON OZONE THERAPY. International Meeting of Ozone Therapy Schools. Madrid, Spain, 2010.
36 Atuma C, Strugala V, Allen A, et al. The adherent gastrointestinal mucus gel layer: thickness and physical state *in vivo*. Am J Physiol Gastrointest Liver Physiol. 2001; 280 (5): G922-G929.
37 Leon OS, Menendez S, Merino N, et al. Ozone oxidative preconditioning: a protection against cellular damage by free radicals. Mediators Inflamm. 1998; 7 (4): 289-294.
38 Barber E, Menendez S, Leon OS, et al. Prevention of renal injury after induction of ozone tolerance in rats submitted to warm ischaemia. Mediators Inflamm. 1999; 8 (1): 37-41.
39 Peralta C, Leon OS, Xaus C, et al. Protective effect of ozone treatment on the injury associated with hepatic ischemia-reperfusion: antioxidant-prooxidant balance. Free Radic Res. 1999; 31 (3): 191-196.
40 Al-Dalain SM, Martinez G, Candelario-Jalil E, et al. Ozone treatment reduces markers of oxidative and endothelial damage in an experimental diabetes model in rats. Pharmacol Res. 2001; 44 (5): 391-396.
41 Candelario-Jalil E, Mohammed-Al-Dalain S, Fernandez OS, et al. Oxidative preconditioning affords protection against carbon tetrachloride-induced glycogen depletion and oxidative stress in rats. J Appl Toxicol. 2001; 21 (4): 297-301.
42 Ajamieh H, Merino N, Candelario-Jalil E, et al. Similar protective effect of ischaemic and ozone oxidative preconditionings in liver ischaemia/reperfusion injury. Pharmacol Res. 2002; 45 (4): 333-339.
43 Ajamieh HH, Menendez S, Martinez-Sanchez G, 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 (1): 55-62.
44 Borrego A, Zamora ZB, Gonzalez R, et al. Protection by ozone preconditioning is mediated by the antioxidant system in cisplatin-induced nephrotoxicity in rats. Mediators Inflamm. 2004; 13 (1): 13-19.
45 Ajamieh HH, Berlanga J, Merino N, et al. Role of protein

synthesis in the protection conferred by ozone-oxidative-preconditioning in hepatic ischaemia/reperfusion. Transpl Int. 2005; 18 (5): 604-612.
46 Martínez-Sánchez G, Al-Dalain SM, Menendéz S, et al. Ozone Treatment Reduces Blood Oxidative Stress and Pancreas Damage in a Streptozotocin-Induced Diabetes Model in Rats. Acta Farm Bonaerense. 2005; 24 (4): 491-497.
47 Calunga JL, Zamora ZB, Borrego A, et al. Ozone therapy on rats submitted to subtotal nephrectomy: role of antioxidant system. Mediators Inflamm. 2005; 2005 (4): 221-227.
48 Borrego A, Zamora ZB, Gonzalez R, et al. Ozone/oxygen mixture modifies the subcellular redistribution of Bax protein in renal tissue from rats treated with cisplatin. Arch Med Res. 2006; 37 (6): 717-722.
49 Li LJ, Yang YG, Zhang ZL, et al. Protective effects of medical ozone combined with traditional Chinese medicine against chemically-induced hepatic injury in dogs. World J Gastroenterol. 2007; 13 (45): 5989-5994.
50 Re L, Mawsouf MN, Menendez S, et al. Ozone therapy: clinical and basic evidence of its therapeutic potential. Arch Med Res. 2008; 39 (1): 17-26.
51 Mawsouf MN, El-Sawalhi MM, Darwish HA, et al. Effect of ozone therapy on redox status in experimentally induced arthritis. Rev Esp Ozonoterapia. 2011; 1 (1): 32-43.
52 Fernandez Iglesias A, Gonzalez Nunez L, Calunga Fernandez JL, et al. Ozone postconditioning in renal ischaemia-reperfusion model. Functional and morphological evidences. Nefrologia. 2011; 31 (4): 464-470.
53 Carpendale MT, Freeberg J, Griffiss JM. Does ozone alleviate AIDS diarrhea? J Clin Gastroenterol. 1993; 17 (2): 142-5.
54 Tanbouli T, Mawsouf MN, Re L, et al. Effect of Ozone Therapy on Foetoplacental Blood Flow in Hypertensive Pregnant Women. International Journal of Ozone Therapy. 2009; 8: 211-216.
55 Delgado-Roche L, Martínez-Sánchez G, Díaz-Batista A, et al. Effects of ozone therapy on oxidative stress biomarkers in coronary artery disease patients. Int J Ozone Research. 2011; 10 (2): 99-104.
56 Díaz AE, Fraga Y, Soria M, et al. Ozonoterapia en la Parálisis Cerebral. Hospital Pediátrico Provincial. Sancti Spíritus. 2006-2009. Revista Española de Ozonoterapia. 2011; 1 (1): 23-31.
57 Martínez-Sánchez G, Delgado-Roche L, Díaz-Batista A, et al. Effects of ozone therapy on haemostatic and oxidative stress index in coronary artery disease. Eur J Phamacol. 2012; in press.

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