A Technically Feasible Treatment for Peritoneal Carcinomatosis

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SUMMARY - *Peritoneal and pleural carcinomatosis are late complications of a primary tumour mainly developed in abdominal organs. Peritonectomy followed by hyperthermic intraperitoneal chemotherapy (HIPEC) represents a feasible and effective intervention, which however has considerable risks and cannot guarantee the cure. It appears reasonable to propose and discuss an alternative treatment based on intraperitoneal application of ozone either as a gas or dissolved in physiological solution. Light and flexible silastic catheters have been implanted into the peritoneal cavities. Two distinct protocols have been used. Protocol* a) *has been based on five daily successive sessions. For each session the ozone concentration was of 50 μg/mL with a gas volume of 2000-2500 mL, hence a total ozone dose of 100-125 mg has been used. Protocol* b) *used at the morning the insufflations of gaseous oxygen-ozone mixture at low ozone concentrations and in the afternoon the intraperitoneal infusion of a sterile lipid emulsion in ozonated NaCl 0.9% solution (42 °C). All the four patients presenting peritoneal carcinomatosis, ascite and multiple metastasis from colon, ovary and pancreas tumors, showed a prolonged survival after treatment based on protocol* a). *The single case treated with protocol* b)*, even if it is clinically irrelevant, it has been significant only for improving technical details. Ozone, not only possesses direct cytotoxic activity on peritoneal neoplastic cells, but* via *its messengers stimulates a number of important biological activities among which immune-stimulation as well as both activation and up-regulation of antioxidant enzymes. In comparison to chemotherapeutic drugs, ozone displays only a local oxidant activity without multi-organ toxicity. Moreover, it is an inexpensive drug and it is easy to use in precisely defined dosages. Based on these technical data, it is hoped that this paper may interest a group of oncologists for both optimizing the methodology and exploring the most effective scheme of ozone delivery.*

Introduction

Peritoneal (involving both visceral and parietal membranes) carcinomatosis represents the late stage of neoplasms originally present in the ovary, colon, appendix, stomach, pancreas and liver [1,2]. Very rarely it may originate from ovarian cells disseminated in the peritoneum during embryogenesis. Neoplastic cells, through the lymphatic lacuna present within the diaphragmatic peritoneum [3] can also invade the pleural membranes inducing also an abundant pleural effusion. Consequently, it is a fairly common complication of frequent human tumors. On the other hand, both peritoneal and pleural mesothelioma are rare tumors linked to asbestos exposure. Sugarbaker [4] was the first to propose a surgical intervention for removing neoplastic agglomerates spread within the abdomino-pelvic cavity, while Spratt et al. [5] have previously indicated the feasibility of the intraoperative hyperthermic intraperitoneal chemoperfusion (HIPEC). These approaches, although effective, are understandably complex and aggressive because peritonectomy may take 8-15 hours and it represents an imposing stress which may be overcome by patients not over 60 years old. Moreover, HIPEC aims to destroy the remaining cancer cells by intraperitoneal hyperthermic (42-43 °C) perfusion with a physiological liquid containing cisplatin realized by an extravascular double perfusion system activated by peristaltic pumps. The initial surgical cytoreduction is complemented by chemotherapy which can use drugs at concentrations 3-5 times higher than those used for intravenous infusion. In particular, HIPEC is painful and it cannot insure the total destruction of neoplastic stem cells.

After evaluating a number of other approaches reviewed by Barni et al. [6], a valid conclusion is that the combination of cytoreductive surgery

with perioperative HIPEC yields an improved survival as compared with systemic chemotherapy. In selected groups of patients, median survival varies from 25 to 60 months and the 5-year survival ranges from 20 to 45%, while morbidity varies from 20 to 40%. However, it appears reasonable to select patients according to the existing independent prognostic variables [7]. Consequently, in order to also extend the treatment in older or debilitated patients, a new approach based on a strongly oxidant gas which can be locally efficacious in the absence of detrimental effects for the whole organism will be suggested.

Reasons to use ozone

During the last two decades we have evaluated the possibility of using ozone as a cytotoxic agent and a biological response modifier [8]. However, serious evidence that the classical ozonated autohemotherapy is beneficial to cancer patients is lacking because firstly randomized, double-blind clinical trials have not been performed and, secondly because ozone acting on blood *ex vivo* is totally exhausted during the following blood infusion into the donor's patient and it cannot act on either primary or metastatic neoplastic cells present in different organs [9]. On the other hand, since 1980 Sweet et al. have shown that ozone *in vitro* inhibits the growth of a variety of human cancer cells [10]. Such an observation is relevant only for intraperitoneal or/and intrapleural (mesothelioma) neoplastic cells proliferating on these serous membranes, which closely resemble the situation *in vitro* where the stratified cells, overlaid with a thin layer of tissue culture fluid, were exposed for 8 days to the gas mixture composed of ozone (0.8 μg/mL) and aqueous vapour. As it happens on the pulmonary epithelial lining during ozone exposure [11], it must be clarified that this gas does not directly attack the cell membrane because it firstly dissolves in the surfactant film layer and reacts with hydrosoluble and lipophylic antioxidants and unsaturated fatty acids. In spite of this instantaneous reaction, the concentration of the ozone messengers, namely H_2O_2, (among Reactive Oxygen Species, ROS) and toxic 4-hydroxy-2,3-trans-nonenal (4-HNE) as the major lipid oxidation products (LOPs) are locally very high and deleterious for cancer cells [10,12]. On the whole, these cells have scarce antioxidant defences against these compounds able to cause either apoptosis or necrosis. Owing to a brief half-life of ozone messengers, partly quenched by natural antioxidants present in the peritoneal or pleural fluids, it appears necessary to slowly administer the oxygen-ozone gas mixture or ozone dissolved in physiological solution during about 60 min at least twice daily. It needs to be emphasized that H_2O_2 via activation of the NFkB, can also activate resident macrophages, neutrophils and lymphocytes, which can release cytokines such as IFN-γ, IL-1 and TNF-α [13]. Another relevant peculiarity is that both ozone and its messengers do not display multi-organs toxicity because they are rapidly quenched by antioxidants present in plasma and lymph.

The aspects of the paradoxical ozone reactivity and mechanism of action have been extensively reviewed [8] and it can be speculated that ozone used in appropriate dosages may act as a therapeutic agent in peritoneal and pleural carcinomatosis.

Materials and Methods

Ozone administration methods

A few technical aspects need to be examined for the ozone administration in the peritoneal-pleural cavities. After the fundamental patient's agreement, one or even better two light and flexible silastic catheters can be implanted into the cavities under strict aseptic technique by an experienced surgeon. Either a silicone or another ozone-resistant catheter can be used, because rubber or other materials commonly used are not ozone-resistant and can disintegrate. To start with, a typical catheter used for chronic peritoneal dialysis (Braun, Milan) has been used but subsequently a lighter catheter (Vygon, Italy, code 296.10) has appeared more practical. One of us (J.C.P.O.) has used a nasal pharyngeal catheter apparently ozone-resistant.

Ozone administration schedules

One of us (J.C.P.O.) has treated four patients affected by colon, ovary, and pancreas neoplasms presenting ascite with concomitant parenteral chemotherapy and intraperitoneal ozone, with a protocol that is based on five daily successive sessions. The ozone concentration used is of 50 μg/mL with a gas volume of 2000-2500 mL hence a total ozone dose of 100-125 mg. He has informed us that intraperitoneal (IP) administration of 50 μg/mL of gaseous ozone represents a painful technique and therefore it is performed under general anaesthesia in the operating room under continuous control of the anaesthesiologist. In a few patients he has also carried out two successive cycles. Although patients treated with both chemotherapy and ozone showed a prolonged survival, no conclusion can be drawn by a mixed therapy. Moreover, both the ozone concentration and total dose are extreme and may be toxic. For this reason, it was felt that a different preliminary experience ought

to be investigated. Such an approach is based on the experimental groundwork that, in order to induce an adaptation to the stress of ozone therapy, it is better to "start low", slowly increasing the ozone concentration. Thus, the following schedule has been used: a) for the morning (9-10 am), gaseous oxygen-ozone mixture as reported in Table 1; and b) for the afternoon (5-6 pm) with a sterile lipid emulsion in ozonated saline [14-16]. As excessive IP ozone doses elicit undesirable pain, it is preferred not to exceed an ozone concentration of 20 μg/mL and in case of the lipid emulsion in ozonated saline the maximum ozone concentration is of 10 μg/mL.

All the patients gave a full informed consent for the therapy.

Administration modalities of ozone at low concentrations

a) The gas volume (about 98% O_2 and 2% O_3) is delivered as ozone is generated by corona discharge and the concentration is accurately measured in real time by assessing the absorption at 253.7 nm within the Hartley band. Medical oxygen is used and the gas mixture is filtered through a 0.22 μm membrane to prevent infection. Also the volume is precisely measured via a 50 mL syringe operated at an interval of two minutes. The initial volume of 500 mL can be progressively increased up to 2 litres. There is no need to evacuate the exhausted gas because oxygen is slowly absorbed with the advantage that the hyper-oxygenation of the peritoneal microenvironment inhibits the proliferation of neoplastic cells, which thrive only in hypoxia.

The initial concentration is of 5 μg/mL and it is progressively increased of 2.5 μg/mL up to a maximum of 20 μg/mL at the seventh session. A total dose of 40 mg administered in one hour is easily acceptable because ozone instantly reacts with antioxidants and biomolecules present in the peritoneal fluid and therefore its actual concentration remains very low.

b) Ozonation of physiological saline is performed by bubbling O_2-O_3 at the desired O_3 concentration (10 μg/mL) for 20 min, possibly keeping the saline at 42 °C and infusing it at the same temperature together with the extemporaneously mixed lipid emulsion. The mixing allows the formation of H_2O_2 (8-10 μmol), a small amount of dissolved ozone and the end product of the peroxidate lipids that will decompose into hydroperoxide and alkenals toxic for neoplastic cells.

Keeping in mind that the peritoneal surface is about 1.7 m^2, the maximum volume of either gas or ozonated saline can be of 2500 mL, aiming to cover both the pelvic and diaphragmatic surfaces. The infusion of ozonated saline at 42 °C is slowly performed *via* one or two catheters in about 60 min while the patient lies on her/his back. Although ozone is a potent disinfectant, the outmost care must be exercised to prevent bacterial contamination of the catheter. Almost needless to say that the primary tumour or macroscopic neoplastic agglomerates had to be surgically removed for improving the ozone treatment. In order to maintain the potency of the antioxidant system of biological fluids in critical patients, the RDA oral administration of both a multivitamin complex and two doses of N-acetylcysteine (1200 mg daily) is suggested. The slow infusion of either the gas mixture or the ozonated saline does not increase the abdominal pain caused by the carcinomatosis.

Results and Discussion

Five cancer patients have been intraperitoneally treated with ozone. Four patients have been classified at IV-V in a ranking of I-V, and they have been already treated with intensive intravenous chemo-

Table 1 The proposed daily schedule for the intraperitoneal (IP) ozone administration

Day	*O_3 μg/mL*	*O_2-O_3 mixture volume mL*	*O_3 total dose mg*
1	5	2000	10
2	7.5	2000	15
3	10	2000	20
4	12.5	2000	25
5	15	2000	30
6	17.5	2000	35
7	20	2000	40
8-30	20	2000	40

therapy. All patients presented malignant ascite due to colon (n=2), ovarian (n=1) and pancreatic cancer (n=1), classified at IV stage. Moreover, the single patient presented both IP and intrapleural carcinomatosis due to an ovarian tumor. Although the single case is clinically irrelevant, it has been also critical for improving technical details. As a consequence of intraperitoneal ozone application, our patient and the four patients subject of the present preliminary investigation improved, as evidenced by the marked decrease of the tumour markers. Apart from such results, no conclusions can be drawn at the moment. As a matter of fact, while ozone useful actions as blocking some UV rays in the stratosphere or displaying bactericidal activity in contaminated water are well known, its proficiency in vascular diseases and in orthopaedics remains almost unknown in spite of well-documented results [17,18]. On the contrary, the deleterious effects of ozone in the pulmonary systems after chronic inhalation are known by everyone but, as it has been clarified [19], this is due to the monthly cumulative ozone dose active on the alveolar lining volume (only about 30 mL) which contains only a minimal antioxidant capacity. In contrast, the plasma possesses a great antioxidant reservoir. That is the reason explaining why therapeutic ozone concentrations can be safely used in Medicine. While judicious blood ozonation does not yield side effects, it procures [8]:

a) blood circulation and oxygen delivery improvements to ischemic tissue;

b) metabolic enhancements by improving oxygen delivery;

c) cellular antioxidant enzymes upregulation, and HO-1 and HSP-70 induction via the activation of Nrf2 bound to the antioxidant/electrophile response element (ARE/EpRE) in the cell nucleus;

d) a more or less direct toxic effect on cancer cells;

e) mild activation of the immune system and enhanced release of growth factors from platelets;

f) a beneficial improvements of coenesthesis in most of the patients, probably by stimulating the neuro-endocrine system.

As previously mentioned, during the common blood ozonation within the therapeutic range (0.42÷1.68 mM) with the successive infusion into the donor's patient, if in most cases improves the quality of life, it does not block metastatic progression. The same results had been obtained in HIV-AIDS patients [20]. In contrast with other claims, our negative results are now well-understood because ozonation of blood *ex vivo* fully exhaust ozone which cannot become in contact with both tumour cells or even free pathogens in the circulation [21] because protected by the plasma antioxidants. Indeed ozone reacting with blood biomolecules has a lifetime of seconds inducing the release of ROS and LOP messengers, which are the true therapeutic effectors. On the other hand, in the case of a direct ozone administration into the peritoneal or pleural cavities, ozone and mostly its messengers readily can interact with neoplastic cells and resident leukocytes. In such a case it is reasonable to postulate biological effects such as the death of neoplastic cells due to H_2O_2 and aldehydic compounds or *via* the activation of neutrophils and macrophages. Obviously, ozone and its derivatives need to be almost constantly present justifying the daily double administration for at least a month. The evaluation of a number of tumoral markers will permit to decide whether the therapy must be continued or not. Such a long period of hospitalization is comparable with that of peritonectomy and the successive HIPEC. There are a few similarities between the photodynamic therapy reviewed by Barni et al. and the ozone therapy approach but it is felt that the latter can be precisely quantified and may be more effective.

A previous result achieved in normal rabbits implanted with VX2 carcinoma HNSCC tumor cells, treated for 5 consecutive days with IP injection of 160-240 mL ozone (equivalent to 8-12.0 mg) showed that 7 out of 14 were cured [22]. In this study the experimental tumor had been implanted only two weeks before the ozone treatment and therefore the situation does not compare with the slow progression of human tumours. It was expected that ozone injected into the peritoneal cavity may have activated resident macrophages and neutrophils which may have switched on an immune-mediated reaction with prompt tumour rejection. In the control group (medical O_2 only) also 3 rabbits (out of 13) were also cured, suggesting the need to be cautious in interpreting an exceptional results similar to many others obtained in mice with IL-2 or endostatin later not confirmed in humans [23]. In contrast with the experimental tumors in rabbits, human neoplasms, when discovered, have already had time to paralyze the immune system by releasing immune suppressive compounds. On this basis, above all lack of toxicity, it appears useful to evaluate the ozone protocol in IP and intrapleural carcinomatosis, even if today there is a lack of validated clinical efficacy.

In conclusion, while the proposed IP ozone administration appears technically feasible, it is clear that only a controlled randomized clinical trials in human peritoneal carcinomatosis can clarify this matter. It is hoped that this paper may interest a group of oncologists, who can have our enthusiastic help in terms of technical knowledge of ozone concentrations and dosages.

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