

V. Bocci

Institute of General Physiology, University
of Siena, Italy

Ozonotherapy as a Possible Biological Response Modifier in Cancer

Key Words

Ozone · Autohaemotherapy · Neoplasia · Cytokines · Leucocytes · Psychoneuroimmunology

Summary

Immunotherapy can nowadays be considered as the fifth modality of cancer therapy besides surgery, radio-, chemo- and hormone therapy. Immunotherapy can be carried out either by exogenous administration of immunomodulatory compounds such as thymic hormones, melatonin, interferons and interleukins or/and by endogenous activation of the immune system by a multitude of inducers, namely bacterial extracts, lipopolysaccharides, vegetable lectins, mitogens, proteinases, Ca⁺⁺ ionophores, CDs antibodies and oxidative compounds. Indeed the discovery that ozone, by rapidly decomposing in several reactive oxygen species, which can transiently induce neosynthesis of cytokines on lymphocytes and monocytes, has given a novel impetus and a rational basis for using ozonated autohaemotherapy as an immune adjuvant. Re-activation of a suppressed immune system represents a meaningful approach in various immunodeficiencies occurring in chronic viral diseases and in cancer. Ozonated autohaemotherapy should achieve a physiological-like re-activation with practically no side-effects. While there are several anecdotal reports on the useful application of ozonotherapy in cancer, no controlled clinical trials have been carried out so far. We hope that on the basis of a rational, recently established protocol we will be able to clarify advantages and disadvantages of ozonated autohaemotherapy, particularly in elderly patients where palliative monochemotherapy procures a poor quality of life with little therapeutic advantage.

Schlüsselwörter

Ozon · Eigenblutbehandlung · Neoplasien · Zytokine · Leukozyten · Psychoneuroimmunologie

Zusammenfassung

Ozontherapie als möglicher biologischer «response modifier» bei Krebserkrankungen

Neben chirurgischem Eingriff, Strahlentherapie, Chemo- und Hormontherapie gilt die Immuntherapie heute als fünfte Behandlungsart bei Krebserkrankungen. Durchführen lässt sich eine Immuntherapie entweder durch Verabreichung immunmodulativer Substanzen wie Thymushormone, Melatonin, Interferone und Interleukine und/oder über die endogene Aktivierung des Immunsystems durch eine Vielzahl von Induktoren wie bakterielle Extrakte, Lipopolysaccharide, pflanzliche Lektine, Mitogene, Proteinase, Kalzium-Ionophore, spezifische Antikörper und oxidative Verbindungen. Tatsächlich hat die Erkenntnis, dass Ozon – infolge seiner raschen Weiterreaktion zu einer Vielzahl reaktiver Sauerstoffspezies – Lymphozyten und Monozyten zur Produktion von Zytokinen aktiviert, zu einer Rationale geführt und ermutigt erneut, die Ozon-Eigenblutbehandlung als Immun-Adjuvans einzusetzen. Die Reaktivierung immunsupprimierter Systeme stellt einen sinnvollen Behandlungsansatz dar, besonders bei Krebs und chronischen Viruserkrankungen, die mit einem Immundefizit einhergehen. Mit der Eigenblutbehandlung bietet sich praktisch eine Methode einer physiologischen Reaktivierung ohne Nebenwirkungen. Bisher liegen über den sinnvollen Einsatz der Ozon-Eigenblutbehandlung bei Krebserkrankungen nur Fallberichte vor, eine kontrollierte klinische Studie existiert nicht. Wir hoffen nun, mit Hilfe eines auf obiger Erkenntnis jüngst entwickelten Behandlungsprotokolls Vor- und Nachteile der Ozon-Eigenblutbehandlung abklären zu können, insbesondere bei älteren Patienten, bei denen eine palliative Mono-Chemotherapie zu einer schlechten Lebensqualität führt und dies bei nur geringem therapeutischem Effekt.

Introduction

Although a number of haematological cancers are now being treated successfully, the common solid cancers, which are the great majority, continue to be a problem for mankind, and in spite of small therapeutic advances the death rate is slowly increasing (table 1). Indeed, due to the prolongation of the lifespan, the fact remains that overall mortality from cancer has not declined over the past decades [1]. This is not likely to change overnight because a highly desirable improvement of chemotherapeutic compounds, so far too unspecific and toxic, is not foreseeable in the near future. An appropriate cancer prevention campaign, aiming at early detection and the use of an appropriate diet rich in fibers and antioxidants [1, 2], may help up to a point because on the whole smoking is not decreasing and is partly shifted from men to women and to Third World countries.

At least theoretically, immunotherapy, that is the fifth modality of cancer treatment, aims specifically to destroy only neoplastic cells, but unfortunately these cells are poorly immunogenic and diabolically equipped to evade or suppress the immune system. Nonetheless, as it is shown in figure 1, since 1980 a considerable effort is being made in developing new and efficient immunotherapeutic approaches that so far have failed in achieving substantial advances [3–10]. Thus it is not surprising that desperate patients are always looking for other possibilities, particularly in the vast field of alternative or, I would say better complementary medical practices. In June 1995, the National Institute of Health (Bethesda, MD, USA), in the wise attempt to put some order in a very intricate problem, has published a report entitled 'Alternative Medicine: expanding medical horizons'. The present classification, by no means definitive, includes 7 major classes: 1) diet, nutrition, lifestyle changes; 2) mind/body control; 3) alternative systems of medical practice; 4) manual healing; 5) pharmacological and biological treat-

Table 1. Death for cancer and estimated annual incidence of the most frequent carcinomas in the EC between 1978 and 1982

Death for cancer	
Italy	
1988	142,674
1991	150,800
1994	~160,000
USA	
1993	526,000
Estimated annual incidence of the most frequent carcinomas in the EC between 1978 and 1982	
Men	
Lung	
Prostate	
Stomach	
Colon	
Bladder	
Liver	
Oral cavity	
Larynx	
Rectum	
Leukaemia	
Women	
Breast	
Colon	
Stomach	
Liver	
Ovary	
Corpus uteri	
Lung	
Cervix uteri	
Rectum	
Brain/CNS	

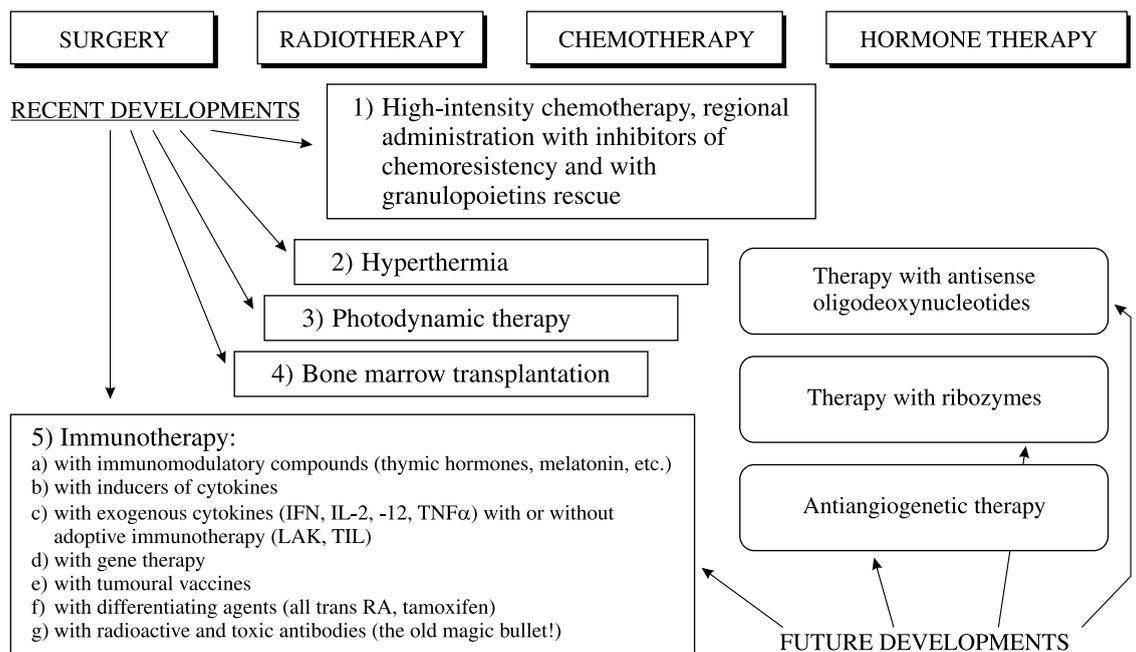


Fig. 1. Conventional antineoplastic therapy.

ments; 6) bioelectromagnetic applications; 7) herbal medicine. On the whole as many as 61 methods have been reported.

The use of oxidizing agents (ozone, hydrogen peroxide) has been included in class 5 among chelation and metabolic therapies, cell treatment and anti-oxidizing agents. Although ozonotherapy is more than 40 years old and has been carried out extensively in central Europe, it has never been accepted by official medicine and is currently strongly despised in England, USA and hardly tolerated in Italy. Several reasons, mostly right but partly wrong, have been previously discussed at length [11–13], and recent experimental data [13–16] from my laboratory, dispel the idea that ozonated autohaemotherapy has become an obsolete therapeutic practice. On the contrary, most of the patients treated with autohaemotherapy according to our procedure report a sense of well-being and euphoria, clearly in contrast with so many patients undergoing hopeless chemotherapy.

Is Ozonated Autohaemotherapy Useful in Cancer?

There are anecdotal reports of major or minor autohaemotherapy suggesting beneficial effects: Beyerle [17] has treated prostate cancer with ‘phenomenal’(?) results. For other types of cancer (throat, ovarian, colon and breast) he comments: ‘We are seeing patients who were bedridden two years ago and sent home to die. They are becoming ambulatory. Their energy level is coming up. They are gaining weight. And we see these spontaneous fractures in the spine are gradually disappearing. Strength is returning to the musculature. There is no spinal pain.’ It is unclear why Dr. Beyerle has not reported the data in a peer-reviewed medical journal, because as presented, they are worthless. Another confusing example was the abstract entitled ‘Ozonotherapy in oncology’, surprisingly selected for presentation at the 12th Ozone World Congress in Lille in May

1995 [18]. If one reads the abstract, it becomes clear why ozone-therapy has such a low reputation in the medical field.

My personal experience is very limited mostly because I have found that oncologists are very reluctant to evaluate ozonotherapy. The only three cases that I had a chance to follow (two terminal lung carcinoma and one metastatic and ulcerated breast carcinoma) did not show, as it was expected, any objective improvement. My feeling is that once the disease has reached the point of no return, any therapy becomes practically useless.

In conclusion, today there is no serious evidence that ozonotherapy can be beneficial to cancer patients because:

- 1) Randomized, double-blind clinical trials have not been performed as they should have been done [19].
- 2) It is unclear whether biological or/and clinical effects, if any, are due to either oxygen or ozone or to both, or simply to blood retransfusion.
- 3) The relevance of the placebo effect is unknown.
- 4) Too often ozonotherapy is carried out together with other conventional or natural therapies so that any result remains questionable.

Another important point that should be kept in mind for future experimentation is which patients are more likely to take advantage from ozonated autohaemotherapy. As I shall discuss in the next section, ozonated autohaemotherapy has a bland immunoadjuvant effect, and therefore it could be more useful in patients with residual minimal disease (after tumour debulking with surgery) than in patients with either extensive metastasis or large tumour load. This concept is exemplified in figure 2, where it is schematically indicated that primary tumours could be either ideally eradicated or more or less extensively removed. The former case is rare because haematogenous dissemination of individual tumour cells (particularly from breast, gastric, prostate and colon cancer) occurs at early stages of the malignancy. Immunocytochemical detection of epithelial tumour cells in bone marrow is now possible and shows that these cells, although they maintain their malignant genotype, usually remain dormant for some time and most of them rest in the G₀ phase [20].

Thus, if only 10³–10⁴ neoplastic cells have been disseminated, there is hope to either destroy them or prevent metastatic growth if the surveillance of the immune system remains active. Today there is a wealth of approaches attempting to achieve this goal (fig. 1); the most promising appear to be immunotherapy and the use of antiangiogenic agents such as endostatin [21]. However, all these approaches are still very experimental and it may take several years before they are validated. Meantime, why not use ozonated autohaemotherapy that has been tested already millions of times and that may exert useful biological activities to prevent tumour progression?

An Evaluation of Possible Mechanisms of Action of Ozonated Autohaemotherapy in Cancer

As indicated in table 2, several biological mechanisms can be activated during ozonated autohaemotherapy. I will briefly discuss each one of them:

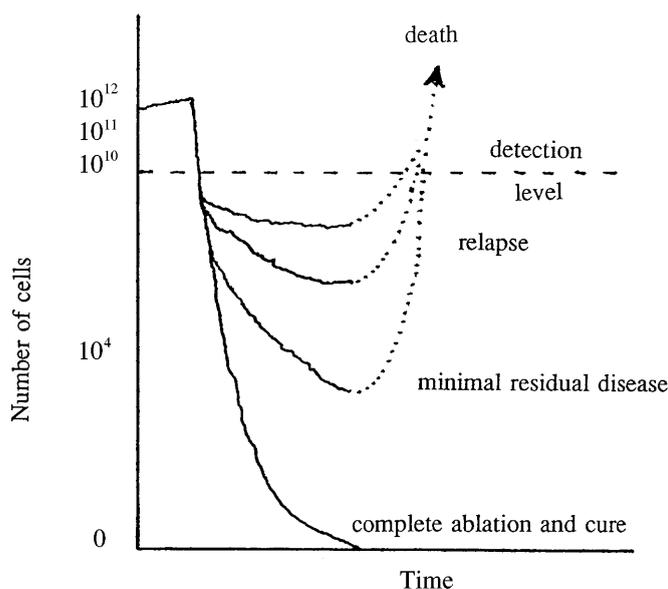


Fig. 2. Tumour mass reduction by cytoreductive therapy.

Table 2. Ozonotherapy in cancer: Possible mechanisms of action

- 1) Direct effect of ozone and reactive oxygen species on cancer cells in vitro and in vivo
- 2) Improved oxygenation and metabolism
- 3) Potential upregulation of the antioxidant enzymatic system with improvement of the cellular redox potential
- 4) Effects on the immune system
- 5) Effects on the CNS and endocrine system. Is there a psychoneuro-immunologic effect?

1) Direct Effect of Ozone and Reactive Oxygen Species (ROS) on Cancer Cells in vitro and in vivo

It has been shown [22] that the growth of human cancer cells is inhibited by ozone during 8 days in culture, suggesting that cancer cells have an impaired defence system against ozone damage. Indeed, as Warburg established a long time ago, there now is some agreement in the assumption that cancer cells live better in a hypoxic environment and that they only have a rudimentary antioxidative enzymatic system to get rid of ROS. However, it remains uncertain whether this is true for all human tumour cells in vivo [23] and if the presence of lipoperoxides in plasma, upon blood reinfusion, can exert a direct cytotoxic effect on neoplastic cells, often hidden in anatomical sanctuaries and protected by the enzymatic and non-enzymatic antioxidants of the host. Zänker and Kroczeck [24] found that incubating neoplastic cells in the continuous presence of a low dose of ozone (<0.5 ppm) for 24 h is distinctly cytotoxic. Moreover, ozone was able to potentiate the cytotoxicity of 5-fluorouracil (5-FU) and to increase the sensitivity in a 5-FU-resistant colon carcinoma variant. If ozone is really able to increase the sensitivity of cancer cells to cytotoxic drugs in vivo, it will become a precious adjunct for chemotherapy. Surprisingly this lead has not been further pursued, probably because it is not feasible to treat a patient with ozone continuously as we can do with a tissue culture. An overlooked aspect is the possibility that, during the exposure of the patient's blood ex vivo to ozone, circulating neoplastic cells could undergo severe oxidation and become a potential autovaccine. This hypothesis is not too far-fetched because a) tumour cells have been detected in blood [25]; b) they are more sensitive to oxygen and ROS than normal blood cells and, if ozonation is carried out using ozone concentrations of 70–90 µg/g of blood for at least 10 min, they could undergo an irreversible damage. It appears worthwhile to pursue this lead because isolation of tumour cells from the primary tumour is costly, time-consuming, not always feasible and yields only the primordial clone. Metastatic cells such as those present in the circulation are usually less differentiated and ultimately kill the patient.

2) Improved Oxygenation and Metabolism

This is the area that has received more attention in the past and it is intuitive that blood exposed to oxygen-ozone, after re-infusion into the donor, enhances the oxygenation and metabolic activities. Several effects such as the increase of glucose 6-phosphate dehydrogenase, 2,3-diphosphoglycerate (2,3-DPG) and adenosine tri-

phosphate (ATP) concentration have already been demonstrated [26, 27]. The validity of other effects such as the release of nitric oxide (NO) and eicosanoids from endothelial cells is under investigation. The release of TGFβ1, which can act as a trophic factor [28], may explain the more rapid healing of ulcers present in ischaemic diseases and may inhibit tumour growth.

In cancer, a more efficient oxygenation may have a direct inhibitory effect on neoplastic cell proliferation and inhibit neoangiogenesis. Moreover, a metabolic improvement of the immune system may enhance the immune surveillance, possibly reducing tumour dissemination.

3) Potential Upregulation of the Antioxidant Enzymatic System with Improvement of the Cellular Redox Potential

Ozone is a strong oxidizer and decomposes in a matter of seconds when it comes in contact with blood. In comparison to ozone, oxygen, that represents not less than 95% of the gas mixture, is less soluble in water and even with continuous but gentle mixing it takes a longer time to equilibrate with blood. Oxygen fully saturates haemoglobin to HbO₂, and although there is an increase of solubilized O₂ into the blood water, no formation of lipoperoxides has been detected. On the contrary, ozone, once dissolved into the plasmatic water, gives rise immediately to a cascade of ROS generated by the oxidation of several types of substrates which, in decreasing order, are: polyunsaturated fatty acids present in plasma lipoproteins, albumin and cellular membranes, sulphhydryl and methionyl groups of proteins, several reducing compounds soluble in plasma (uric and ascorbic acid, bilirubin etc.), carbohydrates and possibly nucleic acids. That means that ozone is highly reactive and that the whole ozone dose mixed with blood reacts with it in a few minutes. Among ROS, the anion superoxide O₂⁻, the hydrogen peroxide H₂O₂ and the hypochlorite ion OCl⁻ can be generated in variable amounts at certain stages of the reaction. All of these compounds are fairly unselective and can damage blood cell components. This is probably the reason why conventional medicine considers ozonotherapy as a dangerous approach more likely to be toxic than therapeutically advantageous. However, it must be considered that if ozonation of blood is carried out when the ozone dose (ozone concentration and gas volume) per gram of blood is exactly known, the reservoir of non-enzymatic and enzymatic antioxidants is capable of minimizing any possible damage to blood cell components. Throughout the course of therapy the patients may take an oral supplement of a multivitamin complex including at most 150 mg vitamin C and 10 mg vitamin E every day [29]. This will ensure that body fluids have a normal level of antioxidants, irrespective of a possible nutritional or metabolic imbalance. Moreover, a wealth of endogenous compounds such as uric acid, bilirubin, melatonin [30], transferrin, haptoglobin, ceruloplasmin, thioredoxin are very efficacious in neutralizing ROS and binding ionized metals, thus preventing the formation of the hydroxyl radical OH[·]. I would like to point out that an excessive supplementation of vitamin C (1 g or more daily) may actually reduce the effectiveness of ozonotherapy and possibly enhance the formation of OH[·].

I have emphasized before [13] that during the ozonation procedure a 'calculated' oxidative stress must occur in order to generate a

certain amount of ROS, particularly H_2O_2 , that act as a crucial signalling molecule for eliciting biochemical and immunological responses. This means that the oxidative stress ought to be strong enough to trigger signals above the threshold level (as otherwise they would be ineffective), but susceptible to be abated in a very short time by the antioxidant system. If this interpretation is correct, ozonated autohaemotherapy is a procedure that does involve a 'calculated', very transient oxidative stress capable of inducing cellular responses with negligible, if any, side-effects. Indeed, as extensively discussed elsewhere [13], these side-effects have never been noted. Thus, we should not be afraid to carry out ozonated autohaemotherapy with an optimized procedure because it does not represent a *chronic* oxidative stress, as it occurs during our lifetime due to ROS continuously generated by about 3% of the breathed oxygen. Furthermore, the exciting novelty is that ozonated autohaemotherapy, repeated twice weekly for at least one month, stimulates the increase of cellular anti-oxidant enzymes such as superoxide dismutase [13, 31] and glutathione peroxidase [26], probably inhibiting the oxidative stress. In retrospect, this finding is not too surprising because in a hyperoxygenated environment a cell either programmes its own death or survives by upregulating the expression and activity of antioxidant enzymes. This interesting new phenomenon of oxidative stress adaptation may be able, at least in part, to explain why ozonated autohaemotherapy has a therapeutic activity in ischaemic, degenerative, auto-immune diseases and possibly in cancer where a persistent oxidative stress has been noted as a factor favouring the progression of invasion and metastasis [32].

4) Effects of the Immune System

It is obvious that, due to its strong disinfectant effect, ozone kills bacteria, viruses, fungi, etc., thus facilitating their phagocytosis by leucocytes. The next step was to understand how ozone activates both the humoral and the cell-mediated immune system. We discovered that ozone acts as an inducer of cytokines; it is known that other oxidizing agents, in appropriate amounts, induce the synthesis of cytokines in monocytes and lymphocytes [33]. It appears that the generated H_2O_2 , an uncharged molecule, crosses the cell membrane freely and activates the cytoplasmic gene-regulatory nuclear factor kappa B, ultimately causing the transcription of mRNAs of several cytokines [34, 35]. On the basis of the findings that the production of several cytokines, namely interleukin (IL-1, 2, 4, 6, 8, 10), tumour necrosis factor (TNF- α) and interferon (IFN β and γ), is very small and transient, it appears unlikely that induction occurs via stimulation of a membrane lectin [reviewed in 12]. In fact the use of a mitogen (PHA) that persistently activates a cascade of protein kinases allows, in the same experimental conditions, the synthesis of cytokines in amounts 100-fold higher than when using ozone. Similarly the proliferation index of blood mononuclear cells (BMC) hardly increases after blood exposure to ozone. This interpretation is in line with the results of Schreck et al. [36], who found that human lymphocytes can express specific mRNAs after a transient exposure to 30–100 $\mu M H_2O_2$.

It has taken several years of research to understand how ozone

Table 3. Why are so many autotransfusions needed to achieve a minimal reactivation of the immune system?

The system comprises about 10^{12} cells dispersed in the BALT, GALT, SALT, spleen, lymph nodes and lymph pool, bone marrow and thymus. Moreover, 1% of these cells are replaced every day by virgin cells.

Ozonation (70 $\mu g O_3/g$ blood or 21 mg O_3) of one bag containing 300 g blood at best stimulates $\sim 6 \times 10^8$ cells, i.e., only 0.06% of the total cell number.

Thus, after ~ 50 sessions (twice weekly for 6 months) we may have activated $\sim 3 \times 10^{10}$ cells equivalent to 3% of the cell pool.

However, microenvironmental release of cytokines may amplify the process several fold. If necessary, more intensive clinical strategies already devised can be applied.

works and why even a small activation of BMC can be useful in an immunodepressed patient. If ozone would have acted as a mitogen upon blood re-infusion, due to the massive release of cytokines, we would have noted a frightening clinical response similar to that observed after intravenous injection of lipopolysaccharide, characterized by shivering, hyperthermia, hypotension, malaise and occasionally coma [5]. Moreover, the disadvantage of a serious flu-like syndrome that few patients are ready to tolerate is associated with the disruption of the cytokine network that is usually deleterious.

In contrast, ozonated autohaemotherapy, even if carried out twice in rapid succession comprising a total of 600 g of blood, never causes any side-effects or, very rarely, a transient tiredness usually followed by a sense of well-being. Nonetheless activated BMC, after homing in their microenvironments, may prime or stimulate neighbouring cells, thus slowly upregulating the immune system. Obviously a single autotransfusion has very little effect, and I would like to propose a protocol including at least two sessions weekly (300 g blood exposed to a dose of ozone of 21 mg for each session, i.e., 70 $\mu g/ml$ ozone per blood) continued for 6 months. Even a simplistic calculation (table 3) justifies this way of thinking: The immune system comprises about 10^{12} cells dispersed in various organs, but for each treatment we at best can transiently activate only about 6×10^8 cells of which we do not know the lifetime and the pattern of re-distribution. It is useful to recall that in the course of adoptive immunotherapy, a critical number of about 10^{10} lymphokine-activated killer (LAK) cells have to be re-infused after exposure to IL-2 *ex vivo* [37, 38]. The scope of this paper is indeed to stimulate interest in this field and evaluate, with controlled clinical studies, if a comprehensive, physiological-like activation of both cellular and humoral immune arms in conjunction with improved oxygenation and normalization of the redox system is useful in neoplasia.

5) Effect on the CNS and Endocrine System. Is There a Psychoneuroimmunologic Effect?

On the basis of many casual observations, a functional interaction between the nervous system, the endocrine glands and the immune cells has been suspected for decades. Recent immunological and

neuroendocrinological studies [39, 40] have clearly shown that these three apparently distinct systems are indeed highly integrated. This topic is beyond the scope of this review, but I cannot omit to comment about the pleasant feeling of euphoria and well-being reported by the majority of patients with chronic hepatitis and age-related maculopathies during autohaemotherapy. Will it occur also in cancer patient? Does ozonated haemotherapy trigger a cascade of endocrine secretions, namely of corticotrophic releasing hormone (CRH), ACTH, cortisol, dehydroepiandrosterone (DHEA) growth hormone, endorphins, melatonin etc.? Could this effect occur due to the withdrawal of a large blood volume or to the re-infusion of ozonated and oxygenated blood with the stimulatory effect of lipoperoxides on the vast surface of the endothelial bed?

It is not difficult to envisage that a change of the homeostatic balance is bound to evoke a multiorgan response that could positively influence the psychological status of the patient, hence the immunological response [41].

Another important question is whether ozonated autohaemotherapy should be carried out in the morning or in the afternoon. Intuitively I would favour the afternoon (4–8 pm) because the normal circadian rhythm ought to be least disturbed [42]. However, only experimental data can define which is the optimal time of the day.

Conclusions and Perspectives

A few anecdotal reports that ozonated autohaemotherapy may give favourable results in cancer are encouraging, but not convincing. Only unbiased, randomized, double-blind clinical studies for several cancer types, possibly carried out in several oncological institutions, can ultimately prove whether ozonated autohaemotherapy is really useful. Due to the lack of serious biological and clinical research in the past, ozonated autohaemotherapy remains in limbo today and is totally disregarded by conventional oncology. This is very unsatisfactory, mostly because in spite of small progresses death mortality remains sustained and real break-throughs are not yet in sight.

There are several, albeit hypothetical, reasons for pursuing the evaluation of ozonated autohaemotherapy as a procedure able to

stabilize the progression of neoplasia particularly in elderly patients more susceptible to the serious side-effects of high-dose chemotherapy. In the last few years I have made a considerable effort to explain that the ozonated haemotransfusion approach has a rational ground and can be carried out in a scientific and reproducible fashion: The ozone dose can precisely be adjusted to the blood weight, and the optimal ozone concentration has been defined, based on experimental data and not on a homeopathic belief [13]. Almost needless to say, a lot of basic work remains to be done, particularly in order to define the molecular and immunological modifications of leucocytes upon ozonation. However, only reliable clinical data can inform us about the validity of the approach because we often have observed that an improvement of immunological parameters is not necessarily paralleled by a complete response and prolonged survival [5, 9].

What will be the future of this approach?

It looks unpromising unless we carry out controlled clinical trials; at the moment, on the basis of my frustrating experience, I doubt very much that we will be able to perform them, due to the disinterest and scepticism of the oncologists. Although I have a great admiration for the scientific strides in biology and medicine, I feel that the biased attitude of most oncologists towards ozonotherapy is wrong and unjustified. On the other hand, most physicians performing ozonotherapy in private practices are unable to perform a clinical trial and unfortunately often use homeopathy, magnetotherapy and similar therapies simultaneously, thus making any conclusion impossible. Consequently, for the sake of the patients, the only hope is that serious and concerted efforts will be made in the next years. For the moment, however, it seems that for lack of dialogue and cooperation a potential therapeutic advantage will continue to remain in limbo to the despair of the patients.

Acknowledgements

I am very grateful to my wife for linguistic revision and to Ms Patrizia Marrochiesi for great care and patience in preparing several drafts of the manuscript. I would like to acknowledge the partial support of the MURST fundings (40 and 60%).

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