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Ozonization of Blood for the Therapy of Viral Diseases and Immunodeficiencies. A Hypothesis

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Abstract—In the last 3 decades major autohemotherapy after exposure to ozone has been used in Europe in uncontrolled trials carried out in patients with many illnesses, particularly chronic viral diseases and neoplasms. It appears that the treatment may activate the host's immune system by inducing the production of immunoreactive cytokines and it may now be possible to rationalize the procedure, improve the regimen and assess the outcome. It is apparent, however, that such a therapeutic approach, in order to be acceptable, requires an investigative effort of biologists and clinicians. Once this is done, owing to the large range of medical applications and the simplicity of the procedure, autohemotherapy could become very valuable particularly in underdeveloped countries.

Introduction

The use of ozone as an effective bactericidal agent goes back to the First World War but it was only in the late 1950s, after the introduction of a reliable apparatus by J Hänslér, that it achieved wide popularity (1, 2). In recent times ozone has become an important subject either as the UV shield in the stratosphere, or a potentially dangerous pollutant (3) even though it remains a useful disinfectant. What is less known is a specialist medical practice consisting in a safe and rapid treatment of blood with ozone followed by reinfusion in the donor (2), either via the intravenous route (major autohemotherapy or 'Grosse Eigenblutbehandlung'), or via the intramuscular route (minor autohemotherapy or 'Kleine Eigenblutbehandlung').

The purpose of this commentary is to examine the state of knowledge of the somewhat controversial use

of ozone in the therapy of human diseases, pointing out the need for an interdisciplinary effort of scientists and clinicians to answer the many pending questions.

The state of the art

As far as the erythrocytes are concerned it appears that, by simply using a range of ozone concentrations between 2–40 µg/ml per ml of blood, one can modify the biophysical and biochemical properties of erythrocytes and improve the oxygenation and blood circulation of ischemic tissues (1, 2).

Far less studied and understood are the mechanisms leading to clinical improvement in patients (treated with autohemotherapy) with acute or chronic viral diseases (herpes genitalis and labialis, herpes zoster, hepatitis, papillomatosis, AIDS and tumors) (2, 4).

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Table 1 reports the few open studies carried out in the last decade. Although all the authors claim highly beneficial results the studies are impossible to evaluate because they have not been published in extensive form. At the Ozone World Congress in 1991 Konrad (5) also reported a 10 year favourable experience with ozone in viral diseases without appropriate controls.

In all likelihood the direct virucidal action of ozone on blood has a negligible role because ozone and peroxides decompose very rapidly and the 200 ml blood sample represents a minimal part of the virus reservoir in the body.

More effective mechanisms may be the enhancement of oxygen utilization with increased metabolism and particularly the stimulation of immunological mechanisms such as enhanced immunoglobulin (Ig) production and activation of phagocytosis (4).

So far, and at least in Europe, the hemotherapeutic treatment has remained in limbo, mostly because it has been and is being carried out as a private practice. Several factors, particularly the empiricism of the procedure, the operator's variability, the lack of well defined parameters (Table 1) and above all of randomized controlled clinical trials have impeded the assessment of the benefits and risks of the treatment. Using good medical practice, risks and side-effects appear minimal if any, but clinical efficacy is based on anecdotal reports, or personal experience, or at best open studies (2, 5) and therefore does not comply with accepted ethical canons and scientific criteria of good research.

A new hypothesis

The main reason for writing this survey is to analyse whether autohemotherapy may be effective because ozonization of blood acts mostly upon monocytes and T lymphocytes as an inducer, thereafter causing the

release of cytokines namely interferons (IFNs), interleukins (ILs), tumor necrosis factor (TNF) and probably colony stimulating factors (CSFs). This hypothesis was put forward almost 3 years ago when I was casually asked to find a rationale for autohemotherapy in viral diseases. Since then, we have demonstrated that appropriate concentrations of ozone can indeed activate monocytes and lymphocytes either in blood, or in buffy coats or in isolated form (6). Briefly, we have shown that firstly, the window of cell activation with ozone is narrow and that either too low or too high ozone concentrations can be ineffective or toxic. Secondly, that at optimal ozone concentration IFN γ , TNF and IL-6 are released in consistent amounts in the incubation medium or in plasma (6, 7), and thirdly that the cytokines are biologically active. These findings have opened a new horizon and give a rational basis for understanding the mechanisms of action of hemotherapy in viral diseases (Table 2). It is well known that IFN γ and to a small extent also TNF, elicit antiviral properties (8) but, most important, these cytokines can stimulate an array of immune functions (8) such as activation of macrophages and neutrophils, increased expression of major histocompatibility complex (MHC) Class I and II on infected cells, monocytes and lymphocytes, enhancement of cytotoxic activity either MHC-restricted, or antibody-mediated, or aspecific via natural killer (NK) cell activation, thus leading to a revival of crucial immune functions previously depressed for genetic, sexual, hormonal, nutritional reasons or senescence (9). It is very likely that several other cytokines such as IL-1, -2, 6 and 8 are released and work in this direction is in progress.

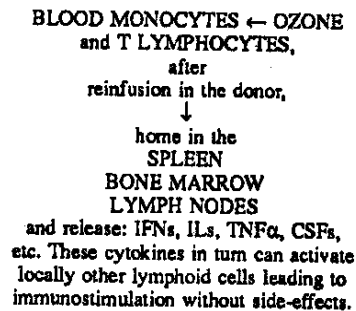
But owing to the fact that IFN α and β are already on the pharmacist's shelf, someone can argue that it is simpler and scientifically more sound to administer a certain dose of IFNs rather than going to the bother of

Table 1 Viral diseases treated with major autohemotherapy

Disease	Blood (ml)	O ₃ (μ g)	O ₃ /Blood (μ g/ml)	Frequency of application	References
Herpes simplex & Zoster	50	800	16	1-2 every 5-6 days	Konrad (1981)
Idem	200	10 000	50	idem	Matassi (1984)
Acute Hepatitis	50	9 000	180	1 every 3 days	Dorstewitz (1981)*
Chronic aggressive Hepatitis	220	8 800	40	1 every week or 2 weeks	Kief (1983)*

* Reported in (2)

Table 2 A schematic view of immunostimulation after major autohemotherapy



collecting some blood, ozonizing and reinfusing it in the donor without knowing exactly which drugs are, or will be elicited and what will be the pharmacodynamic and clinical responses. This certainly is an important problem but one should abstain from concluding that exogenous administration of cytokines is better than active induction, as antisera are no more important than vaccines.

Ozone is an almost ideal cytokine inducer

There is now the exciting possibility of being able to induce in a controlled and reproducible fashion the production of cytokines in immune cells so that upon blood reinfusion these can, by various mechanisms, amplify the response in vivo. In comparison to the single administration of IFN that represents only one lymphokine, which displays side-effects (10) and is not always beneficial (8), the administration of ozonized blood may trigger a physiological cascade of events with a more comprehensive activation of the immune network resulting in a good therapy index. Since the beginning of this century when Coley began injecting bacterial extracts, i.e., endotoxins (that we now know are eliciting release of TNF) and occasionally in a few lucky patients noted tumor regression (11), we have dreamed of finding an ideal inducer that should be atoxic, non-antigenic, non-tolerogenic and yielding a positive immune response without adverse effects (12). Although the search has been active, for many reasons the results have been disappointing (12) and today we do not yet have such an agent. If used with caution and under controlled conditions, ozone could become a cytokine inducer able to fulfil almost all of these requirements.

What needs to be done?

The time has come when we have the technical tools to appropriately evaluate this approach in molecular,

biochemical, immunological and clinical terms. More than a multidisciplinary approach, it is important not to have prejudice against an unorthodox practice that could be beneficial, inexpensive and practically harmless.

There are a number of pressing questions to answer:

Firstly, as blood is in itself a very heterogeneous mixture of components, it is necessary to standardize the ozonization procedure to yield the maximal induction with minimal peroxidative damage to the cells. In order to achieve this, it may be worth while exploring whether the intracellular increase of reduction potential enhances the production of cytokines. It is reasonable to expect that every individual blood will respond differently to ozone induction and in the IFN field we know already the existence of 'good' and 'poor' responders (9, 13). Particularly blood of aged or immunodepressed patients may need to be fortified with reducing agents before ozonization.

Secondly, the evaluation of cytogenetic, phenotypic, proliferative and functional changes of the leukocytes after ozonization may yield valuable insights into what may occur when these cells are returned to the donor. Indeed a third line of investigation should aim to understand the fate of the cells in vivo, particularly their blood clearance rate, distribution and homing behavior. While it has become reliable to label lymphocytes by using Indium-111 Oxine (14), I am surprised that so far, the survival in vivo of human erythrocytes has not been evaluated after ozonization. Nothing is also known about the fate of platelets after ozonization and therefore the question arises about the immunomodulatory role of these cells and/or their products. Moreover platelets (as well as monocytes and neutrophils) are a rich source of prostaglandin E₂ and transforming growth factors (TGFs) beta, both potent suppressor for NK and lymphocyte activated killer cells (15, 16) suggesting that, depending upon the degree of ozonization, blood volume and cellular constituents, hemotherapy may function as either an immunosuppressive or an immunostimulating agent. In practical terms, ozonized blood deprived of platelets and neutrophils, or of monocytes and lymphocytes may act better as either an immunostimulator or an immunosuppressor agent, respectively.

It is tempting to speculate that activated lymphocytes and monocytes home in lymphoid microenvironments (spleen, bone marrow, lymph nodes) and there release cytokines (Table 2). These, by a paracrine mechanism (17), activate neighbouring or circulating cells which in turn can either in-

fluence other cells or, by virtue of circulation, can activate distant sectors of the immune system. We know that this amplification system is operative and normally alert (8, 9, 18) and probably needs to be boosted in patients with viral diseases. It is interesting to note that this process occurs with a minimal leakage of cytokines in the general circulation (9, 19) or, in other words, cytokines in these circumstances do not act as endocrine hormones and therefore side effects are minimal, in fact as it occurs after major autohemotherapy.

This topic introduces the fourth indispensable line of investigation to be performed in patients where humoral parameters (levels of cytokines, acute-phase proteins, complement factors, Igs etc), immunological markers (2', 5'-oligoadenylate synthetase, β 2 microglobulin level, neopterin, IL-2 soluble receptors, to cite a few) leukocytic pattern and activity ought to be measured along with standard hematocemical and enzymatic parameters throughout the procedure. Probably the measurement of genetic markers such as the expression of cytokine genes for IL-2, IL-3, IFN γ , TNF etc will become the most sensitive and informative assay.

A fifth issue concerns the best time of the day to perform hemotherapy and this is generally chosen according to the specialist's and patient's available time; however, from a chronodynamic point of view this is not necessarily correct. Actually, on the basis of our experience with IFN (20), it appears more meaningful to practise hemotherapy in the afternoon than in the morning.

Table 3 Viral diseases that may benefit from major autohemotherapy

- Acute and particularly chronic viral hepatitis (A, B, C, D)
- Recurrent herpes labialis and genitalis
- Herpes simplex keratitis
- Herpes Zoster
- Viral (cytomegalovirus, parvovirus, etc) infections (particularly in immunocompromised patients)
- Papillomavirus infections:
 - recurrent respiratory papillomatosis;
 - genitals and anorectal condylomata (genital warts)
 - epidermodysplasia verruciformis (particularly when evolving to skin cancer or 'Bowen's disease')
- Recurrent respiratory diseases either in children, or in aged people with defective production of IFN or immunological ageing, respectively
- Chronic fatigue syndrome
- Common cold and/or rhinovirus infections
- Mycosis fungoides or cutaneous T cell lymphoma (HTLV-5)?
- AIDS and AIDS-related Kaposi's sarcoma (HIV)

Finally, there are encouraging but uncontrolled findings of hemotherapy in viral diseases and Table 3 reports a list of diseases that could be beneficially treated. Thus it becomes now urgent to design controlled trials possibly in several clinical centers. This is the only way to ascertain whether hemotherapy fulfills the promise and it is the only way to remove this medical treatment from the scientific limbo, no longer an acceptable status for an approach that has been and is being used in thousands of patients every day.

Conclusions

Retransfusion in the donor of blood after ozonization is a medical practice used in Europe during the last 3 decades to cure several types of diseases, in fact too many diseases, while mechanisms of action, at the very least, remain obscure.

A new hypothesis, already substantiated by experimental data, suggests that ozone and secondary reactive species can trigger the production of cytokines in leukocytes, therefore leading to a modulation of the host's immune system. As ozone is a toxic agent, the window of activation without cell killing is narrow but further research may define the optimal dose of ozone for achieving either immunostimulation, or immunosuppression. If autohemotherapy can be understood on a scientific basis, it may become an acceptable practice in orthodox medicine.

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