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Molecular Aspects and New Biochemical Pathways Underlying Ozone Effects

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Abstract

In recent years, emphasis and attention have been focused on the use of medical ozone (O_3). Despite its huge diffusion, certain confusion still persists concerning its potential toxicity as a strong oxidant. Although its use as safety remedy in the medical field has been described since the last century, this confusion still represents a major factor preventing its full acceptance. Furthermore, the use of ozone therapy (OT) in specialities so different like neurology, orthopaedics, internal medicine, sports medicine, dermatology, endocrinology and others makes difficult the collocation of OT as a single specialist branch. In this context, the apparent heterogeneous network of diseases in which OT seems to be active resembles those proposed for the nuclear factor (erythroid-derived 2)-like 2 (NRF2) pathway. This fact, extremely exciting by a pure pharmacological point of view, may cause conflict between the different fields of application and the various medical areas. To our opinion, with the aim to explore the complexity of the OT therapeutic activity in the medical field newly clinical protocols must be designed.

Keywords: Ozone therapy; NRF2; Oxidative stress

Introduction

Although the uses of O_3 in medicine are described since from the past century with excellent clinical results [1,2], only in the recent decades OT has risen to the general attention due to its huge diffusion as medical practice worldwide.

OT is described as one of the best treatment for disc inter-vertebral pathologies [3] with a well-documented recognition by the official science [4-6]. Regarding the therapeutic action on disk herniation, we must always remember that the O_3 effects are mainly due to its main action on the bio-humoral environment [7,8] and only partially by its ability in reducing the size of the herniated disk. Behind this effect, in recent years attention has been focused on the use of medical O_3 in pain management [9-11]. Furthermore, it has been reported its action as an immune modulator and activator of cellular metabolism which shows long-term anti-inflammatory effects reducing inflammation with an apparent low toxicity [12]. Other data demonstrated that OT increases the endogenous antioxidant system activities in endotoxic and septic shock models [13]. However, so far there is only one study aimed at identifying the possible molecular mechanisms of the anti-allodynic/hyperalgesic effect of O_3 and the possible involvement of some inflammatory and apoptotic pathways [14]. Moreover, evidence that antioxidant enzymes, nitric oxide pathways and other sub-cellular activities can be modulated by low O_3 doses is now proven and could support the surprising effects of O_3 in many illnesses [15,16] and other conditions like lung transplantation model [17]. The intention of this short review is to introduce some novel pharmacological concepts on O_3's action. A more detailed and complete report on the full pattern of OT has already made by Noel L. Smith recently [18]. In their excellent review, they conducted a high comprehensive review on OT, investigating its contraindications, routes and concentrations of administration, mechanisms of action, disinfectant properties in various microorganisms, and its medicinal use in different pathologies.

Mechanism of action

Regarding the pharmacological characterization of the O_3 molecule, in the light of the more recent knowledge, we can consider this gas as a pro-drug. Indeed, at adequate non-toxic doses, O_3 can induce the modulation of some biochemical pathways with the activation of second messengers in a cascade with a multiple system actions. Ischemic preconditioning represents the best similarity in this context. Noteworthy, the report of Wentworth et al. [19]. Indeed, their scientific data demonstrate the physiological presence of an O_3-similar mediator during inflammation, indicating O_3 as a new bio-molecule with striking effects that must be considered and studied following new strategies with newly constructed randomized-standardized clinical studies. In addition, a large number of inflammatory and signalling substances, such as tumour necrosis factor and interleukins (interleukin-1beta, interleukin-6, and interleukin-8) [20,21] could be involved explaining the heterogeneous O_3 action.

Looking at the more recent scientific discoveries, allow us to introduce a new terminology regarding the pharmacological mechanism of action underlying the O_3 effect. Indeed, being quite different from a drug, we can't explain its action as a simple interaction between a molecule and a receptor - according to classical schemes of pharmacology currently spread in the medical faculty -but rather as a "Hormetic Stress" [22].

The scientific conviction that a molecule like O_3, i.e. a strong oxidant, could induce benefits in various ailments when used at low doses was described as an unconventional theory. Recently we could note that this concept gained a certain scientific credibility. Indeed, in a recent paper [23] the Nobelist Dr James Watson proposed an unconventional view on oxidative stress and diabetes. The conventional view is that oxidative stress causes insulin resistance. In

his paper, Dr Watson suggested that: "The fundamental cause, I suggest, is a lack of biological oxidants, not an excess", he says. This hypothesis certainly needs to be tested. It could be that it's the balance that matters, and that disease results due to an imbalance on either side; i.e. both oxidative stress and oxidative deficiency (such as hypoxia), could lead to insulin resistance or other diseases. The idea that a conditioning stress induced by small and adequate O_3 doses, similarly to a brief physical exercise, could be helpful in many pathological conditions in cells that burn oxygen to produce energy is now worthy of the scientific community attention. To our opinion new and well-designed scientific studies must be dedicated to the matter taking into account the patient as an integrated whole and not as a simple organ or symptom target. The novel science of Nutraceutics shows similarities on the mechanisms activated by the organism as a whole following a correct supplementation of food [24,25].

Anyway, one of the issues raised by the scientific community is: how O_3 really acts on Humans? As already stated, ozone is quite different from a drug and its action is not a consequence of a binding reaction between one molecule (drug) and one receptor (cellular membrane protein). The fact makes more difficult the efforts of scientists in the attempt to evaluate the molecular events underlying its clinical activity. For the above reasons the O_3 action can't be considered according to classical schemes of pharmacology and new concepts must be defined. To our opinion, reactions induced by stress or hormetic mechanisms require the introduction of a third parameter in addition to the DOSE and the EFFECT: i.e. the TIME. In fact, differently, from what happens for a conventional drug that acts on a specific target with an immediate effect, the stressing agents promote several biological modifications through a myriad of interactions that involve many cellular processes and metabolic pathways that in turn induce a stable clinical effect only after a certain time. Like other Xenobiotic, agents not recognized in the metabolism of the body (from the Greek Xenos = Foreign and Bios = Life), such as heat, mechanical trauma, ionizing radiation or the same foods that we eat daily, even "O_3" molecule is able to influence the cellular functions. Indeed, following an adequate stress, cells promote defence mechanisms with the aim to protect from the specific damage induced by the same stressing agent. The term xenobiotic has been introduced only recently [26], and the details are still lacking adequate scientific support regarding the involved mechanisms. As it is obvious, this fact relegates OT in a limb making understandable, but not reasonable, the lack of attention of Health Authorities usually devoted to the regulation of human health procedures.

O_3, like other agents, and unlike the common drugs that act on a specific receptor, induces small stress to the whole cell when used at adequate doses. This, in turn, triggers a series of intracellular metabolic processes and promotes a myriad of intracellular activities. Because of these reactions, the defence mechanisms of the cell are alarmed and pushed to improve cell activity, explaining in part the surprising therapeutic actions of this gas. A recent study fully explained the biochemical pathways and the intracellular mediators (Transducers, Sensors and Transcription Factors) activated by the different species of xenobiotic (Figure 1) [27].

Pathway	TF	Sensor	Major transducers
Oxidative stress	Nrf2	Keap1	MAPK, ERK, p38, PKC
Heat shock response	HSF-1	Hsp90	CaMK2, CK2
DNA damage	p53	MDM2	ATM, JNK, Chk1, Chk2
Hypoxia	HIF-1	VHL	p38, PI3K
ER stress	XBP-1, ATF6, ATF4	BiP	IRE1α, S2P
Metal stress	MTF-1	None	PKC, CKII, TKs
Inflammation	NF-κB	IkB	IKK
Osmotic stress	NFAT5	None	p38, ATM, PKA

Figure 1: Basic components of major stress response pathways [27].

There is no doubt that this complexity makes us understand why it is not an easy to set up clinical trials demonstrating definitively a possible therapeutic activity of these agents. On the other hand, most of the drug interactions are easily measurable and statistically standardized because of a receptor-dependent action. Briefly, the Oxidative Stress modulates its action through the regulation of the NRF2 pathway [28] which in turn activates some Target Genes modulating proteins synthesis that promotes several cell functions, strengthening the cell defences and optimizing the underlying specific functions.

In a recent study [29] carried out with the O_3 Major Auto-Hemotherapy (MAH) protocol on healthy volunteers, we demonstrated the involvement of the NRF2 protein *in vivo*. Indeed, the levels of NRF2 in peripheral blood mononuclear cells were found to increase immediately after O_3 exposure ($P<0.01$). This effect was still detected ($P<0.05$) in total circulating PBMC when measured 30 min following reinfusion demonstrating that the oxidative stress was able to activate all the blood components. After a series of three MAH's, NRF2 returned to the basal level. At the end of the experiment emerged the late response to OT and an increase ($P<0.05$) of the activities of superoxide dismutase (SOD) and catalase (CAT) were reported by the

authors. These data demonstrate for the first time *in vivo* the activation of the NRF2 pathway by a low O_3 dose followed by the promotion of the proteins synthesis that collectively favours cell survival. We can now better understand why we can observe so different effects after OT. Indeed, this metabolic pathway is common to all the cell lines (Figure 2).

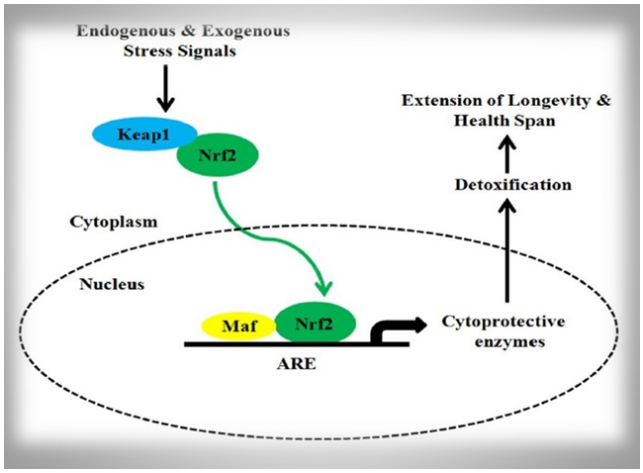

Figure 2: Cellular stress response pathway.

Conclusion and Remarks

Taking into account these last papers, we can conclude that O_3 could be very helpful as integrative and complementary support for pharmacological therapy modulating the oxidative stress component in many illnesses. Furthermore, it could be emphasized its use in the elderly, where side effects and therapeutic costs are going to be even often a serious problem for the Health Authorities and for the medical care personnel [30].

Considering all the above, we can propose OT as a useful resource to complement and integrate the pharmacological approach actually utilized both for the most common symptoms and for rare diseases, still orphan of proper medical treatment [31].

We believe that it is time for the scientific community to launch a serious assessment of OT that can rightfully be considered a strategic ally of the orthodox medicine, especially in the case of rare diseases still orphan of adequate drug treatment. A key factor of no small importance is also represented by the absence of important side effects, the incidence of which, considering the millions of patients submitted to this therapy all around the world over the last 40 years, it is represented by a number after the decimal point preceded by at least 5 zeros. Moreover, in the auspices that this medical therapy could play a primary role in the prevention and in the treatment of the elderly population, we think that a careful assessment and regulation of the OT could no further be delayed. Indeed, the proper education and training of the medical and paramedical staff who will administer the OT according to the most recent clinical indications approved by the international scientific societies like WFOT must be fairly ruled by the Health Authorities worldwide.

Finally, it is our opinion that OT, like other similar holistic approaches, needs of further and deep interdisciplinary studies following the Systems Medicine Approach [32]. Due to the increasingly elderly population, the aim will be also to broaden the knowledge in the field of integrated and natural therapies in the aim of preventing the damage of age and most of the painful pathologies of the elderly.

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