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artículo original

Retinitis Pigmentosa patients treated with ozone therapy during 20 years. Cuban experiences.

Mirta Copello
Retinitis Pigmentosa National Reference Center. "Dr. Salvador Allende" Hospital. Calzada del Cerro Ave. and Dominguez,
(Cerro, Havana, Cuba.)

Silvia Menéndez
Ozone Research Center. POBox 6412,
(Havana City Cuba.)

Contact for questions: Silvia Menéndez. Email: silviamenendez@infomed.sld.cu

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Abstract

Retinitis Pigmentosa (RP) is characterized by bad night vision, decrease of visual field and/or acuity, with electroretinogram (ERG) diminished in amplitude and finally, non reproducible. Taking into account some of the ozone biological effects, as: improvement in the oxygen metabolism, increase in the cell energy, the immunomodulator capacity and enhancement of the antioxidant defense system the aim of this paper is to assess ozone therapy's efficacy in these patients, treated twice a year during 20 years. A controlled and retrospective clinical study was performed in 56 patients with Typical Retinitis Pigmentosa (non-associated), with sample homogeneity, in age, sex and stage of the disease, and with signed informed consent. Forty patients received ozone daily, by rectal way, during 20 sessions (at a concentration of 40 mg/L and a volume of 200 mL) as only treatment, with repetition of this cycle every 6 months, and 16 received other medical treatments (control group). Results showed that, after 10 years, patients in stages I and II had a better response to ozone therapy, and this response was more lasting, with an improvement attained as of 4 months, which is the shortest time period for patients in stages II and IV. After 20 years of treatment, 50.1 % of patients improved their visual field, 31.1 % remained the same and 18 % followed the course of the disease. In respect to visual acuity, we observed that 37.5% of patients improved, 34.9 % remained stable and 27.6 % continued the course of the disease. It is recommended to apply ozone therapy at six-month intervals in order to delay the course of the disease and maintain the visual capabilities. No side effects were observed. Ozone therapy is a good therapeutic choice in the treatment of patients with RP increasing their quality of life.

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Introduction

Retinitis Pigmentosa (RP) is the name for a heterogeneous group of heredodegenerative retinochoroidal diseases of unknown origin, with variable gene expression [1-3]. Its average incidence ranges between 3.2 and 4.5 for every 10000 people in the world, therefore, being an important cause for blindness in the world [2]. It is characterized by initially affecting visual cells and the pigment epithelial layer and by, afterwards, extending to all retinal and choriocapillaris layers. Basically, there are two forms of presentation: typical ones, when degeneration begins in the rods, and atypical ones, when beginning in the cones. It can be found associated to other systemic and ocular diseases, where the presence of cataract, glaucoma, and vitreous and macular alterations can be noticeable. People suffering from the disease lose their visual field and/or visual acuity [1-3].

Patients complain about night blindness and progressive reduction of the visual field, and can even initially present a ring scotoma and, finally, in gun barrel, being noticed that in very advanced stages, in some cases, there can be a loss of central vision. In this disease, electroretinogram (ERG) can appear abnormal or non recordable. [1-3].

Within the physical examination of the eye, it can be observed waxen or pale disks, dimmed or very fine vessels and pigments in the form of osseous spicules in the peripheral means, initially, and, in the most advance stage, it can be distributed to the 4 quadrants. Diagnose is made by exams of visual acuity, visual field, eye fundus and ERG [1-3].

At their beginnings the retinal pigmentary epithelium cells start to [4-8]:
•Remodel its molecular structure and anatomical and histophysiological relations.
•Adapt to new challenges imposed on them by the cell stress, producing this disease, but if the stress increases, an irreversible process begins to develop leading to a cell death.
•Develop neuroplasticity mechanisms, depending on its capacity to obtain diverse growth factors and neurogenic elements, which are necessary in this process.

Hemodynamic studies carried on by researchers have demonstrated the following [4-8]:
-Significant decrease in the blood flow
-Great decrease of vascular diameter.
-Increase in blood viscosity.
-Marked oxygen deficit in the retina.
-Deficit of the necessary nutrients for the metabolism of visual cells.

Normally, these cells need great quantities of oxygen from the choriocapillaris, which is closely related to the photoreceptors, ensuring normal functioning. However, histopathological studies have shown the choriocapillaris is atrophied and disappears within the affected retinian areas [6]. Despite this, there are cell groups resisting prolonged stress, exhibiting a molecular and functional structure apparently normal, which allows them to survive and relate among them [4].

There is not yet a specific treatment to eliminate the cause for production of alteration in the gene. Notwithstanding, some countries have carried on different efforts in searching for therapeutic alternatives to improve the quality of live of these patients [1-3]. There have been several therapeutic assays for the common formularies of RP. But none of them have demonstrated a detention of the degenerative process up to now. It is necessary to continue research in methods to introduce, in the photoreceptors, any substance capable of prolonging the functional and anatomical condition of them, taking into account the genetic pattern mainly. At the moment, current treatments are mainly aimed at diminishing development of alterations provoked by the pathology in the retina [1-11].

In RP, a process to shorten the external photoreceptor segments is produced, initially caused by the

incapacity to adequately use the opsins contributing to the external segment disk formation, redistributing these opsins to the internal segments, unleashing an immunological reaction. The incapacity of the retinal pigmentary epithelium cells to transport the waste elements from the photoreceptor produces [4-8]:

-Free radical formation.
-Changes in the photoreceptor metabolism
-Decrease of oxygenation and glutathione levels in the retinal pigment epithelium.
-Release of citotoxic and genotoxic aldehydes, derived from lipid peroxidation.
-Decrease of the subretinal space.

Taking into account the ozone's biological effects as [12,13]: improvement in the oxygen metabolism, increase in the cell energy, the immunomodulator capacity and increase in antioxidant defense systems, ozone therapy's effectiveness, as only treatment in patients with typical Retinitis Pigmentosa diagnose treated for 20 years in six month intervals treatment cycle, was studied.

Patients and Methods

It was carried out a controlled and retrospective study. This study was accepted by the Scientific Council and Ethics Committee of the Institution, following the principles of the Declaration of Helsinki (1997). All patients gave their informed consent, after receiving the adequate information of the study. The universe was made up of 56 adult patients of both sexes and of different ethnical origin who had been diagnose with Typical Retinitis Pigmentosa and were treated in the National Reference Center for Retinitis Pigmentosa, based in Havana City, Cuba. Forty patients received treatment with ozone therapy, daily (from Monday to Friday), applying, by rectal way, 200 mL of a mixture of gas composed of medical oxygen (O2) and O3 (generated by OZOMED equipments, Habana, Cuba) at a concentration of O3 of 40mg/L, during 20 sessions. This treatment cycle was maintained every 6 months during 20 years. These patients were provided with antioxidant vitamins in intercycles of treatment with ozone therapy.

In the ozone group, patients were classified (10 in each stage) according to the stage of the disease (from I to IV), in accordance with the Cuban Classification of Retinitis Pigmentosa [2]. Patients in stage I presented an initial ophthalmologic manifestation of their base eye disease with visual acuity from 0.6 to 1.0 and visual field with a discrete drop of the peripheral isopters. Patients in stage II had visual acuity between 0.3 and 0.5, with a visual field with pericentral ring scotoma. Patients in stage III presented visual acuity from 0.05 to 0.2 and visual field between 5 and 10 degrees. In this stage, some patients with incipient cataracts, macular alterations of dystrophic type or with glaucoma could be observed. Patients in stage IV presented visual acuity inferior to 0.05 and visual field lesser than 50. This classification was made taking these parameters found in the best eye.

The control group was made up of 16 patients who received other medical treatments as: antioxidant vitamins, electro stimulus [14], dilating vessel medicaments and were allowed to evolve during 20 years.

Improvement Criteria

Visual field (VF), was carried out by Goldman perimeter (it was measured the V4 isoptera and the white stimulus, calculating the area in % and mm2) and visual acuity (VA), applying Snellen chart. It was considered a significant increase of the visual field area by ≥ 25% on the area of basal VF in at least 2 consecutive exams in the year, and in relation to visual acuity, an increase of VA > 2 lines of the Snellen chart.

Results and Discussion

Significant improvement was seen in the variables analyzed, though a group of patients followed the natural course of the disease and whose treatment had been varied according to the cause of this deterioration. It was observed that patients in stages I and II had a better response to ozone therapy (Figures 1 A and 1B) and this response was more lasting in time, with result of a decrease of these effects improvement as of the 4 months, being this the shortest period in patients in stages II and IV.

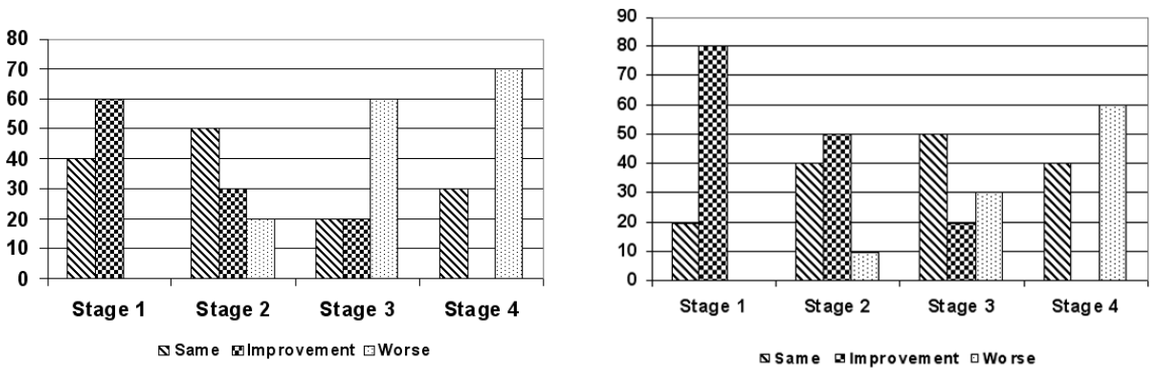

Figure 1. Behavior, with respect to visual acuity (A)and visual field (B) and the different stages of the disease, after 20 years of treatment with ozone therapy

In the cessation in the 10th year (Table 1) it was observed that patients treated with ozone maintain, in relation to the visual field, improvement by 70 % and the 30 % remained the same. In respect to visual acuity, it was observed that 19.0 % of the patients improved, 68.0 % remained the same and 13 % continued the natural course of the disease, with the appearance of macular alterations and presence of glaucoma or cataract, as a typical characteristic of the patient whose disease has maintained its course.

Measurement	Improvement (%)	Same (%)	Worse (%)
Visual field	70	30	-
Visual acuity	19.0	68.0	13.0

Table 1. Behavior after 10 years of treatment with ozone therapy.
Note: Worse-as indicative of the normal course of the disease, not because an impairment due to ozone therapy treatment.

After 20 years of treatment (Table 2), 50 % of the patients improved their visual field, 31.1% remained the same and 18.8 % continued the course of his/her disease. Regarding visual acuity we noticed that 37.5 % of the patients improved, 34.9 % remained stable and 27.6 % continued the course of the disease.

Table 2. Behavior after 20 years of treatment with ozone therapy.

Note: Worse-as indicative of the normal course of the disease, not because an impairment due to ozone therapy treatment.

A significant decrease (p=0.040) of the visual field in the control group in respect to the ozone group was observed (Table 3). The visual field behaviour, for the 20 years of ozone treatment in comparison with the control group, is shown In Figure 2. There is a slow down in the visual field in patients treated with ozone. This is indicative of an increase in the quality of life of these patients.

Table 3. Campimetric results after applied ozone therapy during 20 years.

Group (n=40 patients)	Lost of visual field (%)	Variance
Ozone (24)	9.8	2.08
Control (16)	17.9	4.87
p	0.040	0.045

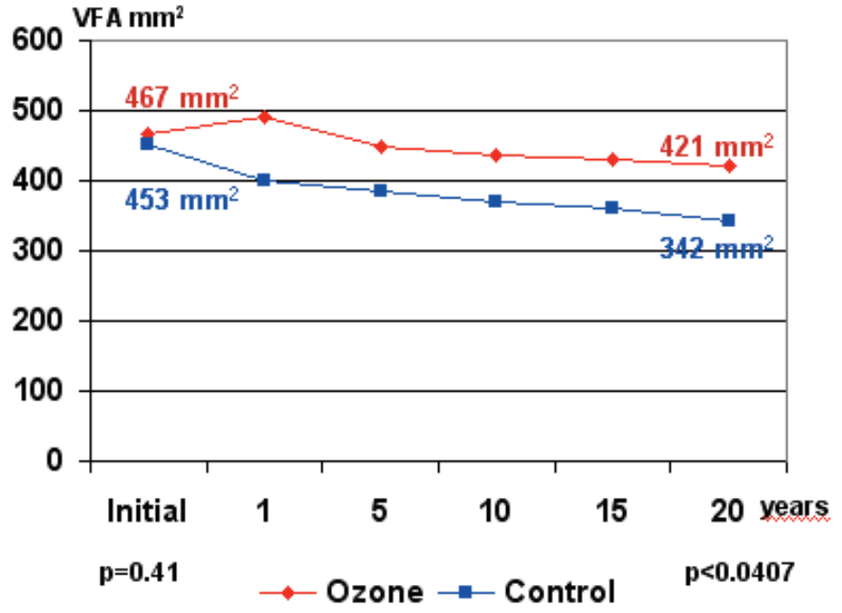

Figure 21 Response to ozone therapy in patients with Retinitis Pigmentosa at long term

The main risk factor for the loss of the retinal ganglion cell (RGC) and its axons is the increase in the intraocular pressure (IOP) (Mechanical Theory), the bad perfusion of the head of the optic nerve (Ischemic Theory) or any hemodynamic ocular alteration above defensive levels of the optic nerve. It is also found the structural injury of the trabecular meshwork and of the juxtacanalicular trabecula, possibly associated an optic nerve ischemia, as well as the decrease in the perfusion with accumulation of oxytocins and glutamate activating the cell death programmed or apoptosis [1.4-6].

Therefore, it is important to preserve the RGC. Among strategies [1.4-6] to prevent death of RGC and justification of the use of ozone therapy are:

-An increase in the cAMP levels. Ozone oxidative preconditioning exerted protective effects against liver ischemia/reperfusion injury through activation of A1 adenosine receptors [15]. Adenosine has been preserved and nitric oxide (NO) level modulated by ozone playing a role in the pathways of cellular signaling which promote preservation of the cellular redox balance, mitochondrial function, glutathione pools, as well as the regulation of the nuclear transcription factor NF-kB and heat shock protein (HSP-70). Adenosine is a major component of vascular homeostasis playing an important role in regulating smooth muscle tone acting via cAMP-mediated cascades to induce vascular smooth muscle relaxation [16].

-An increase of the antioxidant capacity. Ozone may induce an adaptation to oxidative stress or an oxidative preconditioning that under controlled doses, by means of a slight and transient oxidative stress, may stimulate the endogenous antioxidant mechanism, preparing the host to face physiopathologic conditions mediated by reactive oxygen species. This effect has been demonstrated in several papers [17-30].

-The use of inhibitors of nitric oxide synthase (NOS) blocking the peroxynitrite formation. Ozone diminishes the peroxynitrite levels when incrementing activity of superoxide dismutase (SOD), which scavenges the superoxide anion ($O2^{-\bullet}$) [21,31] and, therefore, the following reaction will be seen decreased:

$$O2^{-\bullet} + {}^{\bullet}ON \qquad {}^{\bullet}OONO$$

Peroxynitrite ($^{\bullet}OONO$) is considered as a putative cytotoxin, which has been implicated in the pathophysiology of a variety of processes [32]. It should be pointed out that NO is the only known biological molecule generated in high enough concentrations under pathological conditions to compete and overcome the effects of endogenous SOD for superoxide [33]. The use of i-NOS-specific inhibitor has to be careful because it can increase lipid peroxidation and tissue damage, as it has been referred in hepatic ischemia/reperfusion (I/R) injury [34]. However, NO donor increased the activity of iNOS and decreased the hepatic injury, thus NO production has a beneficial role in hepatic I/R injury [16]. Also, it has to be considered that an excess of NO with a decrease in SOD increases peroxynitrite formation, with a damage effect. In the model of liver injury, ozone prime and activate the genes associated to NOS expression, which promotes NO formation in the required concentrations for protecting against liver I/R injury [31]. Then, a modulation of NO, as ozone is able to produce, is necessary to achieve a positive result.

-The use of antioxidants (to decrease lipid peroxidation). Ozone decreases lipid peroxidation, suggesting the preservations of the membrane integrity. There are several studies (preclinical and clinical) that confirm this ozone effect [17-30].

-To minimize the apoptosis phenomenon. Ozone improves chronic ischemia, one of the causes for apoptosis [12, 13]. Also, in the model of nephrotoxicity after cisplatin application [35], ozone reduced the increase in serum creatine levels and the renal necrosis, inducing a lesser decrease

of the Bax expression (proapoptotic proteins) in cisplatin-treated kidneys. Bax is Bcl-2-like protein that binds and antagonizes the protective effect of Bcl-2 and BclxL, rendering cells more sensitive to death. In this sense, the ratio of expression of Bcl-2 or Bcl-XL to Bax appears to determine cell fate in an adverse environment [38]. Ozone reverted and protected from cisplatin-induced renal damage by modulation of the Bax protein expression, as it can be seen in the following reaction:

Conclusions

It is recommended the application of cycles of ozone therapy every 6 months to slow down the course of the disease and, hence, maintain visual capacities of our patients for the longest possible time, improving their quality of life. This campimetric response is transitory, being more significant in the first 4 months post treatment. The evolutionary stage of the disease influences in the response to treatment negatively, better results are obtained in the initial stages. Non adverse effect was observed during this 20 years of study.

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