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The Role of Free Radicals and Plasmatic Antioxidant in Ménière's Syndrome

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Abstract: The objective of this study was verification, through suitable hematochemical tests, of the supposition that central-systemic microtoxicosis plays a role either in the etiopathogenesis of Ménière's syndrome or in labyrinthine pathological processes or hypoacusis. We did not, therefore, exclude other well-known hypotheses in the causality of these pathologies. Nonetheless, one finds, particularly in the Ménière's cases, a constant homogeneous distribution of the metabolic products of this microtoxicosis, such as a high concentration of free radicals and low natural defenses (e.g., antioxidant plasmatic capacity). Therefore, there exists a kind of dangerous central and systemic presence of reactive molecules, aimed toward the polyunsaturated fatty acids and homeostatic complex enzymes, that is not compensated for by the natural antioxidant defense. The presence of this lack of balance, verified by suitable tests, has shown the rationality of use of a product made from reduced glutathione, thioctic acid, cysteine, and other antioxidants as a multipurpose antidote to this element of etiopathogenesis. Patients were divided into three groups (control, conventional therapy, and antioxidant treatment), and those in the antioxidant treatment group, especially those with Ménière's syndrome, demonstrated a net and more significant improvement. Also, parallel clinical and instrument evaluations of this new therapeutic solution, the efficacy of which has already been positively demonstrated, are expected to provide further evidence to support the primary hypothesis.

Key Words: antioxidant therapy; Ménière's syndrome; microtoxicosis

It is well-known that oxidative stress to which plasma and tissues of the human body—and, in fact, of all living creatures—are subject represents the principal cause of progressive reduction of the organism's functional cellular capacity. In other words, oxidative stress constitutes the main factor of biological aging of all organisms and of cellular degeneration. In recent years, interest in the international scientific literature regarding oxidative stress has been directed also to various pathological situations that attack the human body.

For example, various chronic pathological processes—such as hypertension, dyslipidemia, rheumatoid arthritis, peripheral arteriopathy, and Raynaud's disease—have frequently been shown to be accompanied by a large degree of oxidative stress [1]. Cellular oxidative stress is the result of a continuous conflict inside organisms between the free radicals (which are mainly represented by the derivatives of the reactive metabolites of oxygen, or ROMs) on the one hand and the organisms' total antioxidant plasmatic capacity (APC) on the other.

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IDENTITY OF REACTIVE FORMS OF OXYGEN

Molecular oxygen, for its bi-radical nature, easily accepts unpaired electrons, creating various reduced forms called *reactive substances of oxygen* (ROS). This happens above all at levels of the respiratory chain of the mitochondrion, which shows a difference of potential

at its extremes. The electrons are transported by the cytochrome chain's system to the extremity of this same chain, where the oxygen is reduced to water. However, the oxygen arrives by diffusion at the mitochondrial respiratory system and can easily bind itself to an "escaped" electron, creating a "superoxide radical"; this easily occurs when the breathing chain has a low quantity of adenosine triphosphate, as in hypoxemia [2].

The main intracellular ROS are O, OH, ONOO, and HO, which, owing to their extreme reactivity, are constantly removed by specific reducing metabolic systems. Cellular division, antioxidant mechanisms, or less toxic catabolites reduce the negativity of the ROS. It must be stated that the ROS have specific physiological functions (for instance, in the peroxisome, they provide an antibacterial action), but an excessive production of ROS (not compensated) creates the oxidative stress and the tissue damage, which are the bases of the majority of degenerative processes.

As well as appearing in the mitochondrion, the ROS are created in specialized cells but can be created through irradiation, pollutants, and specific medicines. The mechanisms of extra mitochondrial production are hypoxemia, activation of neutrophils, metabolism of arachidonic acid, and self-oxidation of the catecholamines (formation of adrenochrome).

The effects, sometimes serious, induced by ROS on the cells stimulate us in their individualization, because the very low concentration of these ROS and their short half-life render finding the ROS quite difficult. It is easier to find ROS as a product of their reaction (i.e., the ROMs as cellular aging: synthesis of prostaglandin and thromboxanes and aggregation of platelets, atherosclerosis, Parkinson's and Alzheimer's diseases, carcinogenesis); thus, ROS are most readily identified in incurable patients and in repeatedly hospitalized patients [3].

Oxidative stress can, therefore, be seen as a lack of balance between ROS producers (pro-oxidants) and various protective factors (so-called ROS scavengers) that can be divided into three groups: low-molecular-weight ROS scavengers (vitamin C, vitamin E, carotenoids); derivatives of cellular metabolism (albumin, bilirubin, uric acid); and enzymes that degrade the precursors of ROS (superoxide dismutase, catalase, and glutathione peroxidase) and are found most of all in erythrocytes.

The plasmatic antioxidant capacity that expresses the total protective factors is defined as *total antioxidant plasmatic capacity*, or APC. Vitamin E protects the polyunsaturated fatty acids (PUFAs) from peroxidation; vitamin C regenerates vitamin E. The vitamin E then reacts on the lipidic membranes, and the vitamin C reacts on the cytoplasm or in the extracellular liquid. Also, the myeloperoxidase (neurophils) increases in activity as a defense measure; atoms of iron are pro-oxidants.

The ROS, particularly OH and ONOO, react with all the macromolecules (proteins, lipids, DNA, carbohydrates), generating a second radical that can react with a third macromolecule and so on. Among its most reactive substrates are PUFAs; the proteins are modified in their structure and function; the DNA can see the rupture of a single helix or of cross-links and the substitution of a nucleotide base.

The cells of mammals contain defense mechanisms for detoxifying the radicals (superoxide dismutase, catalase, glutathione peroxidase), and the scavengers interrupt the chain reactions. Vitamin E, enzymes, and such substrates as glutathione are more concentrated where the damage from ROS is more probable. Also, the coenzyme Q10, while stabilizing the membrane potential, acts as anti-ROS, especially in the case of myocardial ischemia [2].

PATHOLOGICAL PROCESSES RELATED TO DAMAGE BY ROS

Several pathological processes are related to damages by ROS, in every organ and in every tissue. Particularly noteworthy are inflammatory symptoms, myocardial infarction, diabetes, cataracts, Down's syndrome, damage from new refusion, heart stunning, damage from ionizing irradiation, and the like. Neurological disturbances, closest to the rationale of this study, derive from the fact that the brain, using more than 20% of the oxygen inhaled, becomes one of the main producers of ROS that attack the high quantity of PUFAs that make up (together with phospholipids) the neuronal membranes. The dopamine is turned into water in Parkinson's disease. In Alzheimer's disease, with the loss of neurons, neurofibrils, plaques, and amyloid, one finds oxidative stress and lack of vitamins C and E in the plasma; an increase of intracellular aluminum causes an increase in lipoperoxidation.

Similarly, in Ménière's syndrome, among the various causes normally recognized, oxidative stress is also postulated. The demonstration of this factor was the objective of this research.

RESEARCH OBJECTIVES

From the international scientific literature, we know that oxidative stress plays an important role in hypoacusis caused by noise, in sudden hypoacusis [4], and in ototoxic cases caused by antineoplastic chemotherapy (especially carboplatin, cisplatinum, and 5-fluorouracil) and from aminoglycosides [5–7]. The ultimate aim of

our research was to demonstrate that in peripheral vestibular abnormalities of a chronic, recurring nature—such as labyrinthitis, cochleovestibulitis and Ménière's syndrome—oxidative stress plays a fundamental and important role in initiating the pathological process and in the recurrence of crisis. We also tried to demonstrate that, by using medicines with high antioxidative powers, we could reduce the oxidant stress in these vertiginous pathologies, thereby reducing the recurrence of the crisis and improving the quality of life of our patients.

SUBJECTS AND METHODS

Subjects

We studied 40 voluntary patients of both genders, aged between 29 and 79 years and suffering from Ménière's syndrome or recurring chronic labyrinthitis (or both). All our patients were evaluated with the ROMs tests and for APC at times T_0, T_1 (after 1 month), and T_2 (after 2 months). During the study, some patients did not follow any therapy (i.e., the control group); others were treated with traditional antivertigo medicines without any antioxidant action; and still others were treated with a new product with high antioxidant powers, made up of an association of reduced glutathione strengthened by its precursor (cistern–SH) and thioctic (or lipoic) acid at a dosage of three tablets per day for the first 15 days and two tablets per day until the second month.

Methods

From the various tests available, for the study we chose the ROMs test and assessment of APC [7]. In the ROMs test (determination of the derivatives of reactive metabolites of oxygen), the dosage of malonyl aldehyde is not always able to show an altered oxidative state. Instead, the elements that first show oxidation are the hydroperoxides that grow from the dehydrogenation action and successive peroxidation by the radical oxyhydrils (OH) on proteins, lipids, peptides, alpha-lipoic, alpha–amino acids, beta–amino acids, and the like. The ROMs test is today the *only* test capable of dosing *all* the hydroperoxides present in a biological sample using the discovery by Fenton in 1894 and Maber and Weiss in 1932, according to which a peroxide, in the presence of a transition metal that acts as catalyzer, generates ROS per the following reactions [1]:

$$Me^{I} + ROOH \rightarrow Me^{II} + RO^{*} + OH^{-}$$
$$Me^{II} + ROOH \rightarrow Me^{I} + ROO^{*} + H^{+}$$

where Me^{I} has a condition of valence and Me^{II} has a superior valence.

A neutralizing molecule of ROS is able to express a coloring from the moment it enters into contact with the ROS, transforming them into ions and assuming a radical, more stable, and photometrically measurable state. The ROMs test measures the oxidative state in true time, dosing in the plasma all the elements that are peroxidated by the free radicals.

The heterogeneity of the peroxidated elements renders it possible to establish a conventional arbitrary unit of measure (UCARR [Carratelli units]) with a scale such as the following:

Healthy subject = 250–300 UCARR
Borderline = 300–320 UCARR
Slight oxidative stress = 320–340 UCARR
Medium oxidative stress = 340–400 UCARR
Strong oxidative stress = 400–500 UCARR
Very strong oxidative stress > 500 UCARR

The APC measures the total natural plasmatic antioxidant capacity as a synergy of the various endogenous systems. The units of measure (micromoles of HCIO per milliliter of serum) associated with each classification are as follows:

Very high reduction < 250 μmol/ml
High reduction = 280/250 μmol/ml
Discreet reduction = 320/280 μmol/ml
Slight reduction = 350/320 μmol/ml
Normal > 350 μmol/ml

One can therefore state that a high ROS and a low APC lead to a pathological state, whereas a low ROS and high APC lead to a functional state. Please note that in the study, to simplify analysis of the data, a conventional index has been adopted between APC and ROMs (APC/ROMs), where the increase of this ratio is an index of rebalancing (reduction) of the oxidative states and a parallel increase of the APC, and vice versa. Also, this index aids in the analysis of percentage tendency.

The patient population has been classified according to various parameters:

Age groups
Ménière's syndrome or labyrinthitis sufferers
$T_0 - T_1 - T_2$
Control group (without treatment)
Conventional therapy group
Antioxidant therapy group
Index (APC/ROMs)
Percentage tendency (APC%/ROMs)

RESULTS

The first datum shown in Figure 1 indicates that at time T_0 in the labyrinthitis patients, ROMs are high, but APC is not highly varied with respect to normal standards. In Ménière's cases, however, as compared to the high lev-

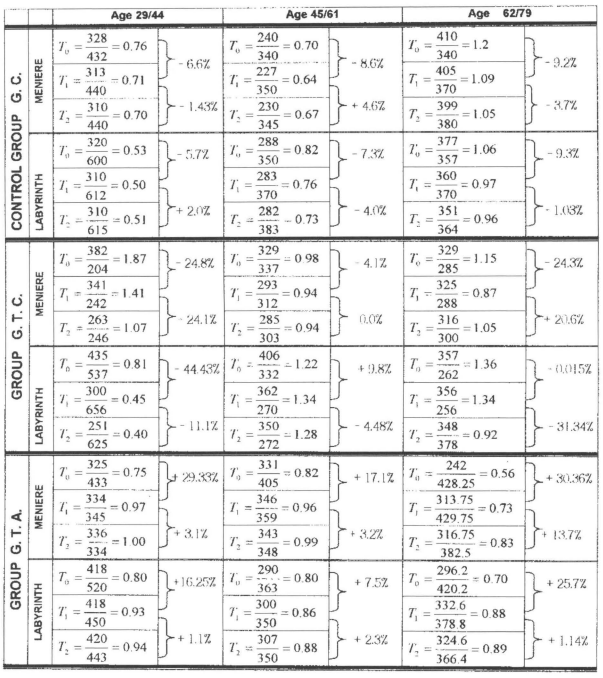

Figure 1. Evidence of ways in which a new hypothesis on the etiological factors of Ménière's disease can shed light on the care of this controversial illness. (*GC* = control group [without treatment]; *GTC* = conventional therapy group; *GTA* = antioxidant therapy group.)

els of ROMs, there are low levels of APC. Thus, the Ménière's cases have a higher degree of oxidative stress than do the labyrinthitis cases.

After the first month of therapy, and therefore at T_1, in the group receiving antioxidant therapy, the Ménière's patients improved more than did the labyrinthitis patients, primarily in the first and third age groups. In the second age group, we noted a brisk but less significant rise in percentage levels (approximately 60% improvement as compared to the other two groups).

The remarkable increases in the percentage levels between T_0 and T_1 in the antioxidant therapy group, registered in both the Ménière's and labyrinthitis patients, is less evident than the increase between T_1 and T_2. Probably this finding is attributable to a reduced dosage of the antioxidant medicine as compared to the dosage administered in the first month of therapy (two tablets instead of three) and to the progressive "saturation" of the physiological APC. It also shows that the antioxidant medicine regulates the APC better than it does the ROMs, which reproduce themselves in every cellular compartment of the organism, evading in part the action of the medicine.

Of note is the fact that the older group of patients also reacts to the therapy in a more continuous and progressive way in the second month (+13.7%), probably because the ratio of APC to ROMs is in a more extreme position with respect to that of the younger population.

In contrast, in the control group, there are no significant differences between Ménière's and labyrinthitis patients, and the percentage tendencies of the ratio APC/ROMs at $T_0 - T_1 - T_2$ have a stationary negative trend.

In the conventional therapy group, particularly in the younger group and in the labyrinthitis cases, the percentage tendencies of the ratio APC/ROMs worsen steadily at $T_0 - T_1 - T_2$, as if this therapy were creating a microtoxicosis with the increased oxidative stress. This finding points up the fact that there is not necessarily a parallelism with the clinical data, as we will see later.

Finally, in the older conventional therapy group of the Ménière's population, there is a wavering increase in the ratio of APC/ROMs, which starts a type of alternating saturation-desaturation of the redox systems.

The first datum that comes to light from the clinical tests is a considerable reduction of symptoms in the antioxidant group both in Ménière's and labyrinthitis patients, particularly in the intermediate age groups (highs of 94% in the Ménière's patients and 83% in the labyrinthitis patients) (Fig. 2). However, if we consider

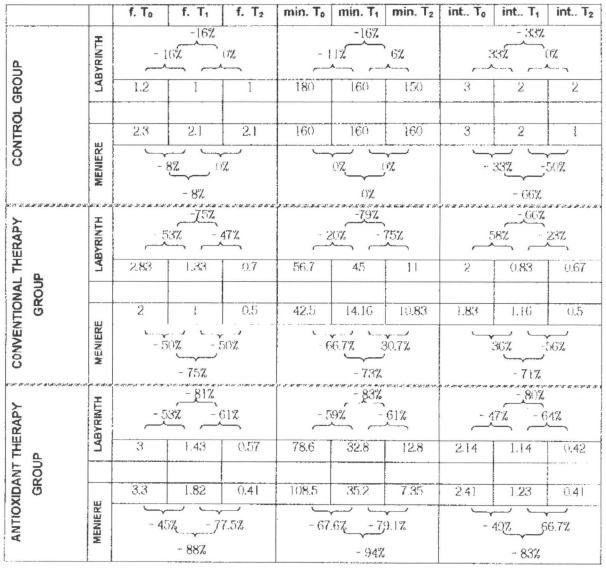

Figure 2. Clinical tests reveal reduction of symptoms in the antioxidant group. (*f* = frequency, or number of crises per month [at $T_0 - T_1 - T_2$]; *min.* = duration of crisis in minutes [at $T_0 - T_1 - T_2$]; *int.* = intensity of symptomatology [at $T_0 - T_1 - T_2$; points awarded according to the following system: 3 = debilitating; 2 = better in bed; 1 = can move, but with difficulty; 0 = wellness].)

as homogeneous the three groups treated with antioxidant therapy, there is an average improvement of 88.3% in the Ménière's group and an increase of 81.3% in the labyrinthitis group. In contrast, in the conventional therapy group, an improvement of 73% in the Ménière's patients and of 73.3% in the labyrinthitis patients is evident.

It is necessary to point out, however, that conventional therapy consisted of several agents, whereas the antioxidant therapy group was rigorously maintained on monotherapy.

For a more complete picture, we have included the group that received no treatment. For these patients, the Ménière's and labyrinthitis cases improved by 24.6% and 21%, respectively. These data demonstrate that these chronic pathological processes, even if left untreated, undergo modest spontaneous remissions over a long period, as part of the natural history of the illnesses.

CONCLUSIONS

Both the hematoclinical and the clinical evaluations prove the rationality of using this high-powered antioxidant medicine—*Gluta-Tios* (Omega Industries) in Italy—in the treatment of vertigo symptoms, especially in Ménière's patients. In those suffering from recurring chronic labyrinthine pathologies, the use of this medicine is particularly important in preventing crises. This clinical field has been discovered because the classic conventional medicines (diuretics, cortisones, etc.), which exhibit a slight effect in individual cases, do not have the capacity to act on the microtoxicosis etiology of this illness, though *Gluta-Tios* does.

From the data on hand, it is clear that long-term therapy with gluta-tios is optimum therapy aimed at preventing recurring crisis in the identified pathological processes. This research, which is only in its third month, will be the object of future scientific communications in which the results will certainly be more significant and refined.

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