Glutathione and Cell Health

International Seminar on Complementary Treatment of Cancer

11-12 March 2016

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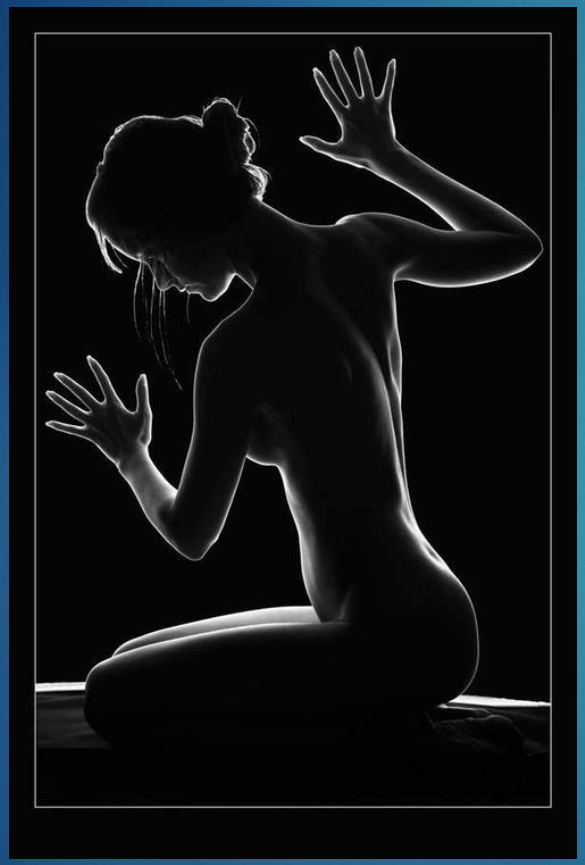
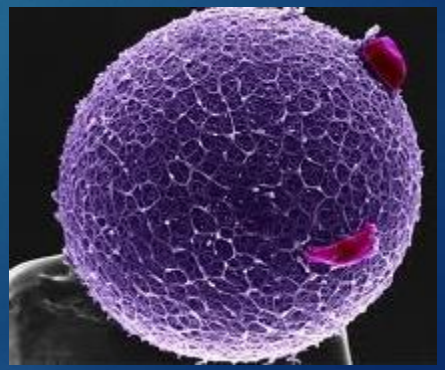


- The human body is a wonderful machine, formed by 20 to 30 trillion cells working in a concerted manner.



Glutathione and Cell Health

- Since the beginning of the industrial revolution have been manufactured more than 112,000 xenobiotics and the propagation of electromagnetic waves has increased.
- Our body does not know what to do with those molecules that day to day we are incorporating into our system.
- There are more than 70,000 artificial chemicals and about one thousand new substance which are introduced into the market every year.
- As we are getting old the levels of Glutathione decreases and our defenses against cancer as well, thus, restoring the levels of GSH, although if the disease is not there, is a good prevention technique.

OXIDATIVE STRESS

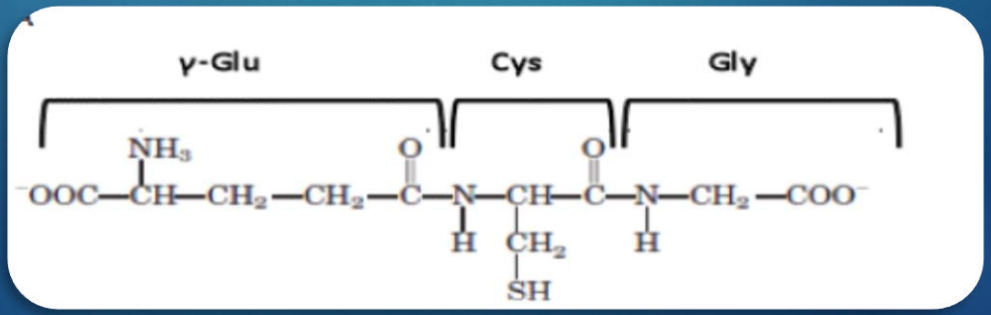
- Oxidative stress accelerates the degenerative processes associated with aging. And it can also promote the formation of tumors by damaging DNA, activating procarcinogens and alter cellular antioxidant systems.
- The cells are then forced to regenerate and telomeres, which are the clock that determine the number of times a cell can replicate, are accelerated due to damaged DNA by free radicals (FR) precipitating programmed cell death (apoptosis).
- Nature is very wise and she balanced things; to neutralize free radicals and detoxify the cells, the body produce a series of antioxidant enzymes, among the most effective and multipurpose is Glutathione.

Glutathione and Cell Health

- The GHS was discovered in 1888 by French scientist Ray-Paihade. Glutathione is not an essential nutrient, as it can be synthesized from the amino acid L-cysteine, L-glutamic acid and glycine.
- The sulfhydryl (thiol) group (SH) of cysteine serves as a proton donor and is responsible for the biological activity of glutathione.
- This amino acid is the limiting factor in the synthesis of glutathione in cells because cysteine is scarce in foodstuffs.
- Moreover, if released, as free amino acid, cysteine is toxic and spontaneously is catabolize in the gastrointestinal tract and blood plasma.

Glutathione biosynthesis

- The tripeptide glutathione is synthesized in the cell cytoplasm by the action of two dependent enzymes adenosine triphosphate (ATP) which is the one who donates energy to both enzymes.
- **First**, gamma-glutamylcysteine is the synthesized from L-glutamate and cysteine through the gamma-glutamylcysteine synthetase enzyme (also known as glutamate cysteine ligase (GCL)). This reaction is the limiting step in the synthesis of glutathione.
- **Secondly**, glycine is added to the C-terminal gamma-glutamylcysteine through the glutathione synthetase enzyme.



Limiting factor in the synthesis of GSH

GAMMA-GLUTAMILCISTEÍNA SINTETASA

L-GLUTAMATO → CISTEÍNA

↓

GAMMA-GLUTAMILCISTEÍNA

(GLUTAMATO CISTEÍNA LIGASA (GCL))

Glutathione biosynthesis

- Glutathione exists in the reduced (GSH) and oxidized forms (GSSG).
- In the process of donating an electron, glutathione becomes reactive, but readily reacts with another reactive glutathione to form glutathione disulfide (GSSG).
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Glutathione biosynthesis

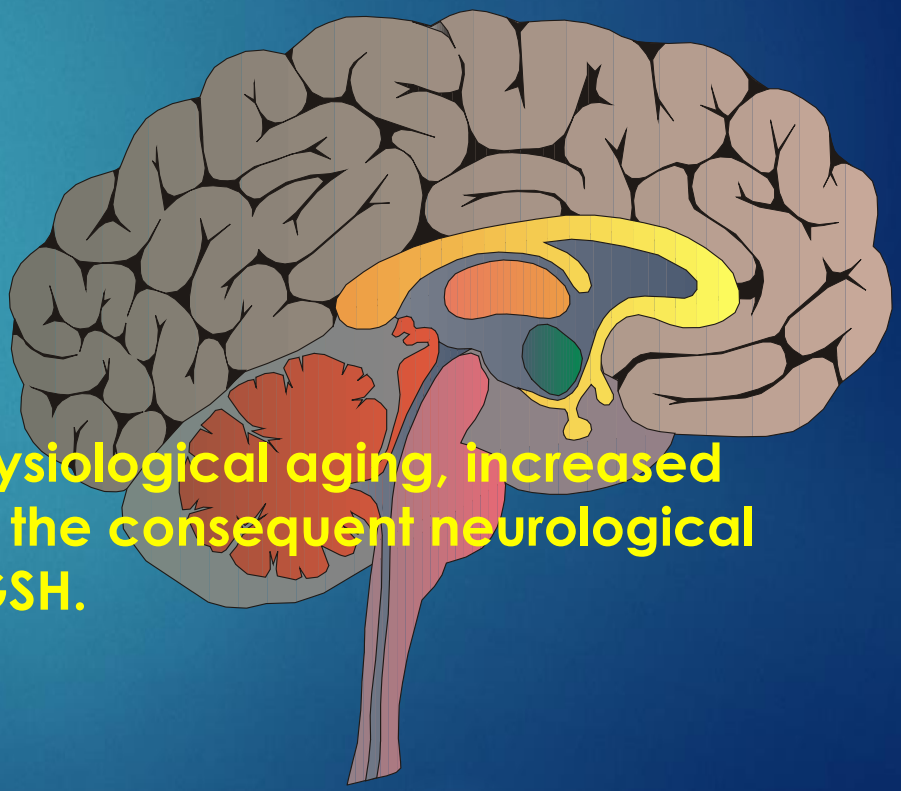
- This reaction is possible due to the relatively high concentration of glutathione in cells (up to 5 mm in the liver).
- GSH can be regenerated from GSSG by the enzyme glutathione reductase.
- In healthy cells and tissues, more than 90% of total glutathione is in the reduced form (GSH) and less than 10% exists in the disulfide form (GSSG).
- Increased ratio of GSSG (glutathione disulfide) and GSH is considered indicative of oxidative stress.
- The normal ratio of GSH and GSSG concentration is 1/10, GSH varying between 1 and 10 mm.

Glutathione biosynthesis

- Antioxidants constitute an axis of protection against reactive species production, these include enzymatic and non-enzymatic type.
- Glutathione (GSH) in its reduced form is a non-enzymatic, and is one of the first lines of defense against oxidative damage.
- The biological functions of GSH involve participation as antioxidant, neuromodulator, detoxifying, so its deficiency is important in the onset of neurodegenerative diseases and cancer.

Central nervous system disease such as:

- Alzheimer
- Multiple sclerosis
- Amyotrophic Lateral Sclerosis
- Epilepsy ezquizofrenia
- Depression
- Cerebro Vascular Accident
- Cerebral ischemia



- They have in common with physiological aging, increased ERO and nitrogen species and the consequent neurological damage with large deficit of GSH.

Glutathione and Cell Health

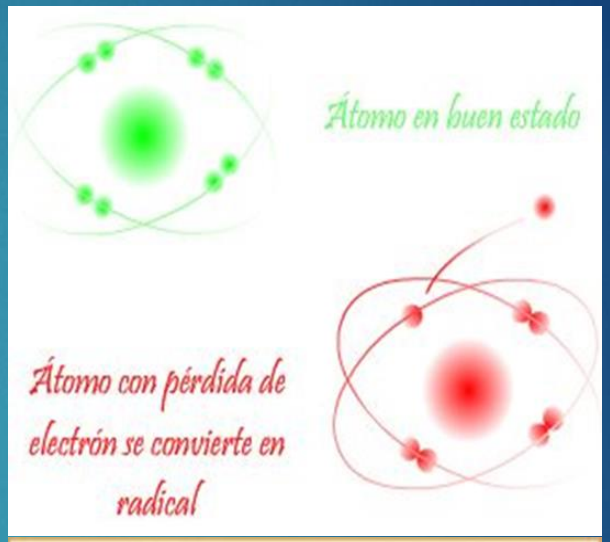
- The concentration of Glutathione on average is 12 mM in mammalian cells.
- It has important functions as an antioxidant and is important part of the detoxification of xenobiotics.
- It is essential for cell proliferation and has an important role in apoptosis, since the decrease in the amount of glutathione allows the caspase activation and the progression of apoptotic mechanisms.
- A very important function is to maintain the oxide reduction potential.

Ballatori N, Krance SM, Notenboom S, Shi S, Tieu K, Hammond CL (2009) Glutathion dysregulation and etiology and progression of human diseases. Biol. Chem 390:191-214.

Franco R, Cidlowski JA (2009) Apoptosis and glutathione: beyond an antioxidant. Cell Death Differ 16:1303-1314

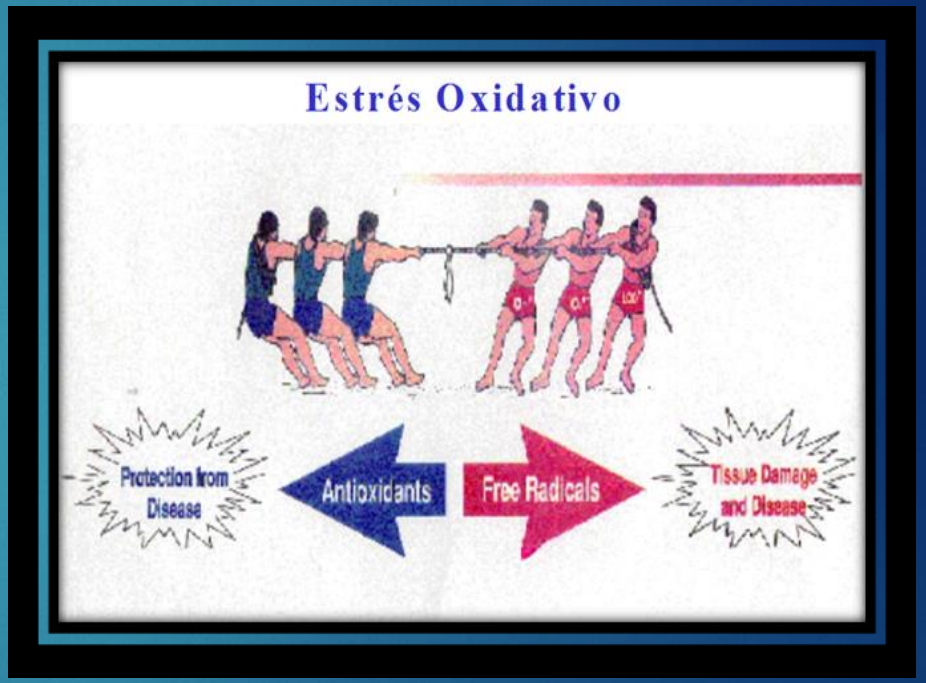
FREE RADICAL

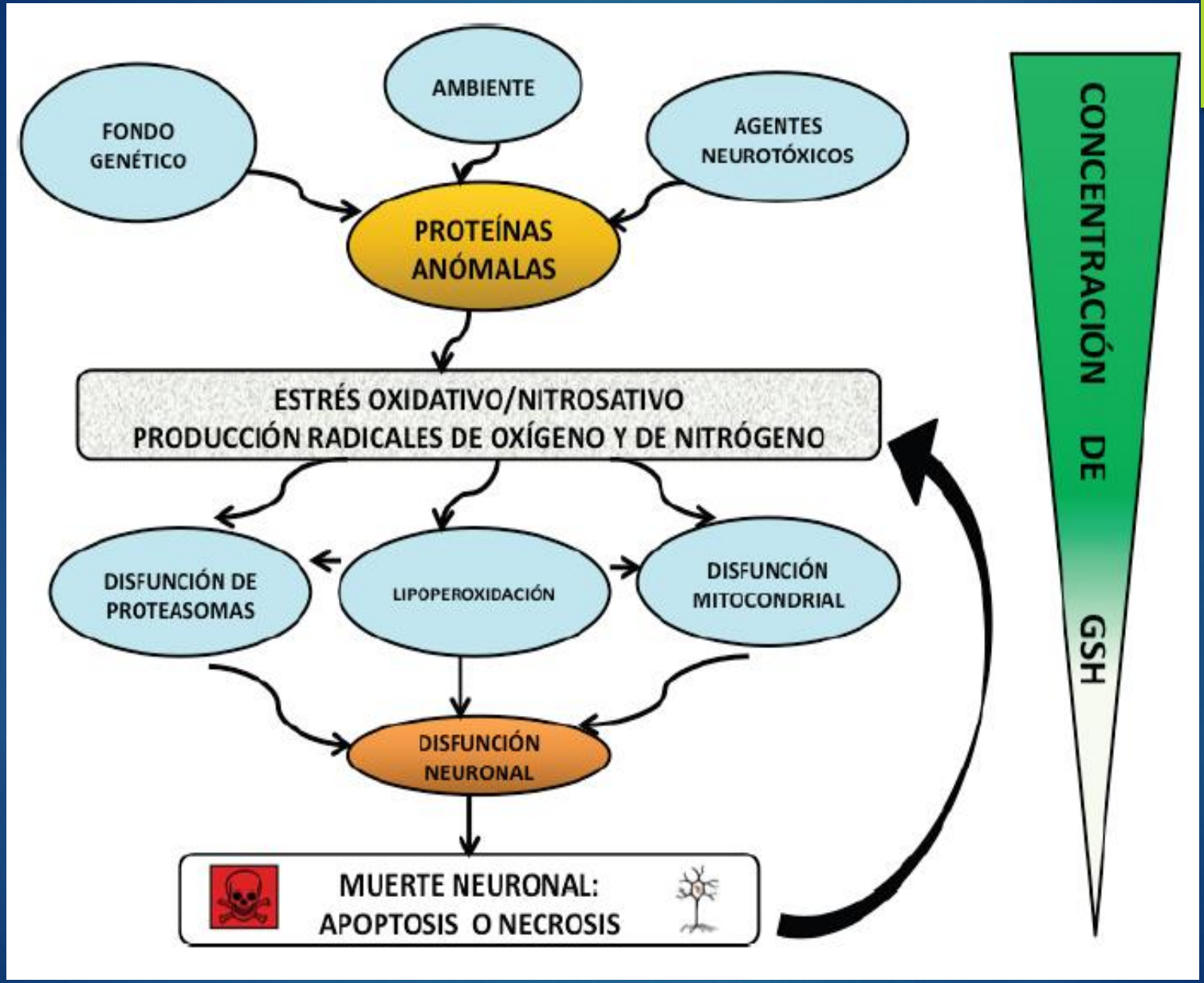
- The free radicals are molecules or molecular fragments with one or two unpaired electrons.
- An unpaired electron increases the chemical reactivity of an atom and seeks to complement its last orbital.
- That is why they have a very short life, (millionths of a second) and are highly reactive with other molecules.



Oxidative Stress

- Oxidative stress is caused by an imbalance between ROS (superoxide anion, hydroxyl radicaless, and hydrogen peroxide) RNS (nitric oxide, nitrogen dioxide, and peroxynitrite) and the antioxidant capacity of the cell.
- The damage these substances cause are related to carcinogenesis, atherosclerosis, neurodegeneration, etc.




AMBIENTE
FONDO GENÉTICO
AGENTES NEUROTÓXICOS
PROTEÍNAS ANÓMALAS
ESTRÉS OXIDATIVO/NITROSATIVO
PRODUCCIÓN RADICALES DE OXÍGENO Y DE NITRÓGENO
DISFUNCIÓN DE PROTEASOMAS
LIPOPEROXIDACIÓN
DISFUNCIÓN MITOCONDRIAL
DISFUNCIÓN NEURONAL
MUERTE NEURONAL:
APOPTOSIS O NECROSIS
CONCENTRACIÓN DE GSH

Characteristic of cancer

The "seal" of cancer

- Self-growth capacity
- Insensitivity to exocellular growth control signal
- Avoidance of apoptosis.
- Unlimited replicative potential.
- Angiogenic capacity.
- Invasiveness and metastasis.
- Glycolytic phenotype (bioenergetics)

Oncological reality

- He has managed to evade the immune system and body control.
- The peritumoral tissue is at the service of the tumor and supplies the necessary nutrients.
- The guest does not acknowledge having a problem.
- It is a silent disease

BIOCHEMICAL CHARACTERISTICS OF CANCER CELLS

Cancer cells to survive necessarily need to change their biochemical characteristics, such as:

- Decreased of the enzyme manganese superoxide dismutase dependent superoxide dismutase (Mn-SOD), leaving copper zinc dependent (Cu-Zn SOD) as the only destructive agent superoxide (O2 • -)
- It has very low levels of catalase and SOD.
- Produce elevated amounts of anion superoxide (O2 • -)
- Inactivation of the rest of Cu-Zn SOD enzyme.

BIOCHEMICAL CHARACTERISTICS OF CANCER CELLS

- The accumulation of large amounts of O2 • - increase the inhibition of catalase, which could becomes depleted, increasing levels of H2O2
- High levels of oxidation of DNA with elevated 8.OHdG
- High rate of lipid peroxidation (as evidenced by elevation 4 hydroxy nonenal (4HNE) DNA break
- Inhibition of apoptosis

PHYSIOPATHOLOGICAL MECHANISMS INVOLVED IN CANCER

- The energy produced in the mitochondria as ATP it does through the process called "Krebs cycle" which requires oxygen to function properly.
- However, cancer cells tend to use a simpler process that does not require oxygen and occurs outside the mitochondria called "anaerobic glycolysis".
- Because cancer cells "turn off" their mitochondria activity (not used for energy), this may be the way of how these cells avoid death and become immortal.

PHYSIOPATHOLOGICAL MECHANISMS INVOLVED IN CANCER

- The dedifferentiation of normal cells toward cancer occurs when they experience withdrawal of 60% or more of oxygen for an extended period of time.
- To survive, they change their energy metabolic pattern of aerobic pathway by anaerobic fermentation of glucose, earning 20 times less energy in the form of ATP (150kJ against 2,870 kJ)
- Most solid tumors have PO2 lower than in normal tissues of origin.

Takizawa S, Matsushima K, Shinohara Y, Ogawa S, Komatsu N, Utsonomiya H, Watanabe K (1994) Immunohistochemical localization of glutathione peroxidase in infarcted human brain. J Neurol Sci 122:66-73

Janaky R, Ogita K, Pasqualotto BA, Bains JS, Oja SS, Yoneda Y, Shaw Ca (1999) Glutathione and signal transduction in the mammalian CNS. J Neurochem 73:899-902

PHYSIOPATHOLOGICAL MECHANISMS INVOLVED IN CANCER

- The fermentation of glucose left as final waste CO2 and lactic acid, generating an important area of acidity.
- The macrophages and T cells interpret the acidity signal as a punctual damage that requires repair.
- Growth factors are then released and stimulate the cells to replicate.
- A greater number of cancer cells, higher acidity, more growth factors, more cancer cells in a vicious circle that will lead to death.

PHYSIOPATHOLOGICAL MECHANISMS INVOLVED IN CANCER

- This lactic acid little by little transforms the internal environment and leads to an acid pH which uses glutamine and lowers the glutathione, at the same time consumes proteins, because they are transformed into glucose via gluconeogenesis, which It serves to feed the tumor.
- A tumor needs 16 times more glucose than normal tissue.

MECANISMOS FISIOPATOLÓGICOS INVOLUCRADOS EN EL CÁNCER

- Cancer is the only disease known in which glutathione level, which is usually low, is very high in the tumor but not in the rest of the body.
- This is probably because the tumor cells is anaerobic and do not perform the tricarboxylic cycle, which is the major free radical generator and therefore do not consume the glutathione.
- Due to this, nitric oxide remains high and causes vasodilation in the blood vessels of tumor-prone to massive hemorrhages.
- The increase in nitric oxide inhibits leukocyte migration toward the tumor cells, which favors their expansion and metastasis formation.

Cancer and GSH

- Many tumor types have high levels of glutathione, factor that makes them highly resistant to chemotherapy and radiotherapy, however, the cancer cells have lost the ability to self regulate its metabolic and growth functions.
- One of the things that the tumor cells do not do well is to regulate the metabolism of glutathione.
- When cancer cells are subject to high amounts of glutathione precursors, or glutathione itself, its production inside the cell go off. This process is called **downregulation**, healthy cells react differently.
- In the same way that lower the levels of glutathione in cancer cells makes them more susceptible to damage, including chemotherapy and radiotherapy, increase levels of glutathione in healthy cells makes them more resistant to these treatments. This leads to have fewer side effects such as nausea, vomiting, diarrhea, hair loss, and leukopenia (loss of white blood cells).

Hypoxia inducible Factor [HIF]

- HIF is a transcription factor that regulate cell response to hypoxia and acts as a regulator of oxygen homeostasis.
- We now know that HIF and hypoxia are major determinants in angiogenesis and besides regulate the processes of invasion and metastization determinants of tumor aggressiveness.
- This active transcription factor gens that encode proteins by increasing the availability of oxygen and allow metabolic adaptation in its absence, controlling the expression of dozens of products involved in angiogenesis, erythropoiesis, glycolysis, invasion, apoptosis, vascular tone, pH regulation, epithelial homeostasis and drug resistance.

Semenza GL. HIF-1 and human disease: One highly involved factor. Genes Dev. 2000;14:1983-91.

Kaelin WG Jr. Molecular basis of the VHL hereditary cancer syndrome. Nat Rev Cancer. 2002;2:673-82.

Hypoxia inducible Factor [HIF]

- These adaptations to hypoxia become the most difficult to treat and more resistance to therapies tumors. This adaptability of hypoxia by malignant cells is critical for tumor growth. Tumor hypoxia by itself is an important epigenetic regulation factor of the protein HIF-1a.

- Besides inhibiting HIF-1a hypoxia generates oxygen free radicals, which are able to stabilize HIF-1a protein's and to induce gens of HIF (hypoxia inducible factor) and VEGF (vascular endothelial growth factor) gene.

Kaelin WG. ROS: really involved in oxygen sensing. Cell Metabolism. 2005;1:357-8.

Muzandu K, Shaban Z, Ishizuka M, Kasusaka A, Fujita S. Nitric Oxide enhances catechol estrogen induced oxidative stress in LNCaP cells. Free Radical Research. 2005;39:389-98.

Strategy against tumor

- Any treatment involves to determine when and how to act.
- Evaluate well the type of tumor and QT
- Neutrophil elevation usually are messengers of the tumor progression.
- I suggest request for an immune profile: CD8, CD4 and NK cells. If they are low I suggest start with a program aimed to modulate the immune system.
- If we have positive ultrasensitive PCR with high TGF-β we can start with an anti-inflammatory program.
- Do not disturb the main treatment. The priority is the destruction of the tumor and not the protection of the host.

Ozone and tumor

- Ozone and ERO are closely related with tumor glycolytic metabolism.
- The treatment of cancers requires a modification in the nutrition with low calories and ketosis.
- Tumor death occurs by apoptosis / necrosis.
- The 1st requires energy and FR, this is provided ONLY by the mitochondria and energy should be supply by the ozone.

Glutathion and Ozone

- If in addition of given glutathione we add ozone applications for several months, bone marrow will be able to induce new generations of "gifted erythrocytes" with increase in the content of 2,3-DPG, antioxidant enzymes as well as elevated glucose 6-phosphate dehydrogenase (G6PD),
- this can allow a profound change in functional activities leading to tumor tissue from hypoxic state to normoxic. If this happens, it will dramatically change the tumor microenvironment leading the neoplastic cells to a very vulnerable state or inactivity.

Bocci V. Oxygen Ozonetherapy. A Critical Evaluation. Kluwer Acad. Press 2002; p.306-314

Glutathione has multiple functions:

- Increases the proliferation of lymphocytes, which increases the magnitude of the response, Increases the activity of elimination of cytotoxic T cells and NK cells, and regulation of apoptosis, thereby maintaining control of the immune response. GSH is responsible for the available Hb to transport O2.
- GSH helps reduce the mucus and inflammation of the airways, especially in obstructive diseases such as asthma and bronchitis. It helps eliminate the RL produced by the combustion of smoking, contributing to the detoxification of the lungs.
- GSH chelates (them inert) heavy metals (mercury, cadmium, arsenic and iron) makes water soluble and thus facilitates their excretion. GSH softens the effects of chemotherapy and radiotherapy.

GSH FUNCTIONS

- With an adequate supply of GSH, patients cope better with oxidative stress induced by HIV or any infections or viral diseases.
- With high levels of GSH some liver functions are restore and improve protection against diabetes complications.
- Protect the harmful effects of acetaldehyde which is the major product of alcohol metabolism and is responsible for most long-term damage.
- GSH helps fight fatigue and Chronic Fatigue Syndrome and fibromyalgia.
- Good health of the crystalline requires high amounts of GSH.
- The liver, heart and lungs also have high demand for GSH.

GSH FUNCTIONS

- The good health of pregnancy and breastfeeding also requires very high levels of GSH.
- At the same time it protects the brain tissue and acts to recycle the Vit E, which has the ability to reduce brain acidity preventing damage causing the FR.
- The brain is the organ most susceptible to attacks by the oxidative products per gram than any other organ, as the tissue demand more oxygen and glucose.
- The bad news is that we produce optimal levels of GSH up to 20 years thereafter it decreases by 10% per year, therefore, our organism is much more susceptible to the attack by FR

GSH FUNCTIONS

- High levels of GSH increases insulin growth factor (IGF-1) Type 1, therefore **decelerates** programmed cell apoptosis.
- It increases the production of DHEA (youth hormone) giving energy to the body.
- Decreases Tumor Necrosis Factor and inflammation.
- Reduces the presence of C-reactive protein involved in inflammatory processes.
- Regulates the activation of NF-KB (nuclear factor kappa enhancer light chains of activated B cells), involved in many chronic and degenerative diseases and immunological development inadequate.

PROTOCOL

- Glutathione we can administer essentially intravenously.
- 600 mg diluted in 50 ml of physiological saline mixed with 1 gr of Vit C.
- The frequency is determined by the individual oxidative stress, (one to two times a week).
- In cancer for example, the frequency will be twice a week, 20 sessions.
- It can be administered immediately after the major autohemotherapy.

WITHOUT GLUTATHION…

- Every cell in your body would die prematurely.
- The entire system (immune) of your body would stop working.
- Your liver, which cleans all the toxins you ingest or inhaled could stop performing this function, since the Glutathione is responsible for detoxification.
- The oxygen-based life (as human), would be impossible.

GLUTATHIONE IS THE VITAL KEY MOLECULE FOR LIFE, HEALTH, PHYSICAL PERFORMANCE AND LONGEVITY OF EVERY HUMAN CELL.

THANK YOU VERY MUCH