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Supplementation with N-Acetyl-Cysteine (NAC) increases the amounts of γ-L-Glutamyl-L-Cystein-Yl-Glycine, which reduces the amounts of Reactive Oxygen Species (ROS) in normal cells and is effective in the Prevention of Cancer

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Abstract

The aim of this study is to mention the effectiveness of Glutathione supplementation for the prevention of cancer. Glutathione (GSH) is an important antioxidant in plants, animals, fungi, and some bacteria and Archaea. As we mentioned in our several researches, the prime cause of cancer is increasing the amounts of ROS in cells which damage the mitochondrial cristae and also due to the butterfly effect, it causes the cells to become cancerous instead of apoptosis. The studies in humans and mice shows the relation between increasing the amounts of GSH in vivo and in vitro, and decreasing ROS level in cells.

Keywords: *Glutathione; ROS; Cancer Prevention*

INTRODUCTION

Glutathione (GSH)

Glutathione (GSH) is an important antioxidant in plants, animals, fungi, and some bacteria and Archaea. Glutathione is capable of preventing damage to important cellular components caused by reactive oxygen species such as free radicals, peroxides, lipid peroxides, and heavy metals [1]. It is a tripeptide with a gamma peptide linkage between the carboxyl group of the glutamate side chain and the amine group of cysteine, and the carboxyl group of cysteine is attached by normal peptide linkage to a glycine. Thiol groups are reducing agents, existing at a concentration around 5 mM in animal cells. Glutathione reduces disulfide bonds formed within cytoplasmic proteins to cysteines by serving as an electron donor. In the process, glutathione is converted to its oxidized form, glutathione disulfide (GSSG), also called L-glutathione [2, 3].

Butterfly Effect and Cancer

The prime cause of cancer is the damage to the mitochondria in normal cells. Nearly all cancer cells contain damaged mitochondria and the basic reason behind this, is increasing the intracellular inflammation or basically the incline in Reactive Oxygen Species (ROS) produced by each mitochondrion in oxidative phosphorylation. Increasing the ROS in a cell can cause damage to the DNA of the mitochondrion and also Nucleus DNA, but another reason behind turning the normal cell into cancer cell is the chaos caused by the increasing of inflammation inside each cell and increasing the intracellular ROS. [S. Zaminpira, S. Niknamian, ECRONICON, 2017] these chaos causes some abnormal messaging between the DNA of the nucleus to stop the apoptosis and turning the oxidative phosphorylation to the fermentation in cytosol. Normally by damaging to the mitochondria, the cell should apoptosis. However; the nucleus sends wrong messages to stop the apoptosis and do fermentation process in cytosol to survive the cell. Even some normal left mitochondria would be shut down and stop the oxidative phosphorylation. This is the main and the real reason how increasing intracellular inflammation can cause cancer. [S. Zaminpira, S. Niknamian, EC Cancer, 2017]

MATERIALS AND METHODS

Once oxidized, glutathione can be reduced back by glutathione reductase, using NADPH as an electron donor [3]. The ratio of reduced glutathione to oxidized glutathione within cells is often used as a measure of cellular oxidative stress [4].

The biosynthesis pathway for glutathione is found in some bacteria, such as cyanobacteria and Proteobacteria, but is missing in many other bacteria. Most eukaryotes, including humans, synthesize glutathione, but some do not, such as Leguminosae, Entamoeba, and Giardia. The only archaea that make glutathione are Halobacteria [5].

Glutathione is not an essential nutrient for humans, since it can be synthesized in the body from the amino acids L-cysteine, L-glutamic acid, and glycine; it does not have to be present as a supplement in the diet. The sulfhydryl group (SH) of cysteine serves as a proton donor and is responsible for its biological activity. Cysteine is the rate-limiting factor in cellular glutathione biosynthesis, since this amino acid is relatively rare in foods [6].

Cells make glutathione in two adenosine triphosphate-dependent steps:

1. First, gamma-glutamylcysteine is synthesized from L-glutamate and cysteine via the enzyme gamma-glutamylcysteine synthetase (glutamate cysteine ligase, GCL). This reaction is the rate-limiting step in glutathione Synthesis [7].
2. Second, glycine is added to the C-terminal of gamma-glutamylcysteine via the enzyme glutathione synthetase [6].

Glutathione exists in both reduced (GSH) and oxidized (GSSG) states. In the reduced state, the thiol group of cysteine is able to donate a reducing equivalent ($H^+ + e^-$) to other molecules, such as reactive oxygen species to neutralize them, or to protein cysteines to maintain their reduced forms. With donating an electron, glutathione itself becomes reactive and readily reacts with another reactive glutathione to form glutathione disulfide (GSSG). Such a reaction is probable due to the relatively high concentration of glutathione in cells (up to 7 mM in the liver) [8].

In healthy cells and tissue, more than 90% of the total glutathione pool is in the reduced form (GSH) and less than 10% exists in the disulfide form (GSSG). An increased GSSG-to-GSH ratio is considered indicative of oxidative stress [9].

Glutathione (GSH) participates in leukotriene synthesis and is a cofactor for the enzyme glutathione peroxidase. It is also important as a hydrophilic molecule that is added to lipophilic toxins and waste in the liver during biotransformation before they can become part of the bile. Glutathione is also needed for the detoxification of methylglyoxal, a toxin produced as a byproduct of metabolism [10].

This detoxification reaction is carried out by the glyoxalase system. Glyoxalase I (EC 4.4.1.5) catalyzes the conversion of methylglyoxal and reduced glutathione to S-D-lactoyl-glutathione. Glyoxalase II (EC 3.1.2.6) catalyzes the hydrolysis of S-D-lactoyl-glutathione to glutathione and D-lactic acid [11].

Function in animals

GSH is known as a substrate in conjugation reactions, which is catalyzed by glutathione S-transferase enzymes in cytosol, microsomes, and mitochondria. However, GSH is also capable of participating in non-enzymatic conjugation with some chemicals[12,13]. Animal glutamate cysteine ligase (GCL) is a heterodimeric enzyme composed of a catalytic and a modulatory subunit. The catalytic subunit is necessary and sufficient for all GCL enzymatic activity, whereas the modulatory subunit increases the catalytic efficiency of the enzyme. Mice lacking the catalytic subunit (i.e., lacking all de novo GSH synthesis) die before birth [14,15]. Mice lacking the modulatory subunit demonstrate no obvious phenotype, but exhibit marked decrease in GSH and increased sensitivity to toxic insults [11,16,17].

While all animal cells are capable of synthesizing glutathione, glutathione synthesis in the liver has been shown to be essential. GCLC knockout mice die within a month of birth due to the absence of hepatic GSH synthesis. [10] Major transport into the blood stream is driven by an electrochemical gradient, specifically through the transport proteins RcGshT and RsGshT [12,13,18]. Similarly, glutathione S-conjugates, synthesized hepatically, feature preferential secretion into bile [12,19].

In the case of N-acetyl-p-benzoquinone imine (NAPQI), the reactive cytochrome P450-reactive metabolite formed by paracetamol (acetaminophen), which becomes toxic when GSH is depleted by an overdose of acetaminophen, glutathione is an essential antidote to overdose. Glutathione conjugates to NAPQI and helps to detoxify it. In this capacity, it protects cellular protein thiol groups, which would otherwise become covalently modified; when all GSH has been spent, NAPQI begins to react with the cellular proteins, killing the cells in the process. The preferred

treatment for an overdose of this painkiller is the administration (usually in atomized form) of N-acetyl-L-cysteine (often as a preparation called Mucomyst), which is processed by cells to L-cysteine and used in the de novo synthesis of GSH [11,15].

Cancer and ROS

ROS are constantly generated and eliminated in the biological system and are required to drive regulatory pathways. Under normal physiological circumstances, cells control ROS levels by balancing the production of ROS with their elimination by scavenging systems. But under oxidative stress conditions, excessive ROS can damage cellular proteins, lipids and DNA, leading to fatal holes in cells that contribute to carcinogenesis.

Cancer cells exhibit greater ROS stress than normal cells, due to oncogenic stimulation, increased metabolic activity and mitochondrial malfunction. ROS is a double-edged sword. On one hand, at low levels, ROS facilitates cancer cell survival since cell-cycle progression driven by growth factors and receptor tyrosine kinases (RTK) require ROS for activation and chronic inflammation, a major mediator of cancer, is regulated by ROS [20]. On the other hand, a high level of ROS can suppress tumor growth through the sustained activation of cell-cycle inhibitor [9,10] and induction of cell death as well as senescence by damaging macromolecules. In fact, most of the chemotherapeutic and radio-therapeutic agents kill cancer cells by augmenting ROS stress [21].

The ability of cancer cells to distinguish between ROS as a survival or apoptotic signal is controlled by the dosage, duration, type, and site of ROS production. Modest levels of ROS are required for cancer cells to survive, whereas excessive levels kill them [22].

Metabolic adaptation in tumors, balances the cells' need for energy with equally important need for macro-molecular building blocks and tighter control of redox balance. Therefore, production of NADPH is greatly enhanced, which functions as a co-factor to provide reducing power in many enzymatic reactions for macromolecular biosynthesis and at the same time rescuing the cells from excessive ROS produced during rapid proliferation. Cells counterbalance the detrimental effects of ROS by producing antioxidant molecules, such as reduced glutathione (GSH) and Thioredoxin (TRX), which depend on the reducing power of NADPH to maintain their activities. [23]

Most risk factors associated with cancer interact with cells through the generation of ROS. Reactive Oxygen Species then activate several and various transcription factors such as nuclear factor kappa-light-chain-enhancer of activated B cells, activator protein-1 (AP-1), hypoxia-inducible factor-1α and signal transducer and activator of transcription 3, leading to the expression of proteins that control inflammations, cellular transformation, tumor cell survival, tumor cell proliferation and invasion, agio-genesis and metastasis as well. ROS also control the expression of various tumor suppressor genes like p53, retinoblastoma gene (Rb), and phosphatase and tensin homolog.

DISCUSSIONS AND RESULTS

Due to the review of the biochemistry of Glutathione in the body of humans and animals, Glutathione has multiple functions:

1. t maintains levels of reduced glutaredoxin and glutathione peroxidase, It is one of the major endogenous antioxidants produced by the cells, participating directly in the neutralization of free radicals and reactive oxygen compounds, as well as maintaining exogenous antioxidants such as vitamins C and E in their reduced active forms. [22,23,24].

2. Regulation of the nitric oxide cycle is critical for life, but can be problematic if unregulated. Increasing the NO in the blood and tissues means increasing the oxygen concentration which is important in the prevention of cancer [25].

3. It is used in metabolic and biochemical reactions such as DNA synthesis and repair, protein synthesis, prostaglandin synthesis, amino acid transport, and enzyme activation. Thus, every system in the body can be affected by the state of the glutathione system, especially the immune system, the nervous system, the gastrointestinal system, and the lungs.

4. It has a vital function in iron metabolism. Yeast cells depleted of GSH or containing toxic levels of GSH show an intense iron starvation-like response and impairment of the activity of extra-mitochondrial ISC enzymes thus inhibiting oxidative endoplasmic reticulum folding, followed by death. As all cancer cells are similar to the function of yeast cells, increasing the amounts of glutathione will increase the rates of apoptosis in cancer cells.

5. It has roles in progression of the cell cycle, including cell death. [5] GSH levels regulate redox changes to nuclear proteins necessary for the initiation of cell differentiation. Differences in GSH levels also determine the expressed mode of cell death, being either apoptosis or cell necrosis. Manageably low levels result in the systematic breakage of the cell whereas excessively low levels result in rapid normal cell death or converting to cancer cells. [14,17].

CONCLUSION

Glutathione is the major endogenous antioxidants produced by the cells and participating in reducing the ROS levels in cells. It increases NO levels, is important in DNA synthesis and repair and also regulate the immune system of the body. Systemic bioavailability of orally consumed glutathione is poor because the molecule is the substrate of proteases of the alimentary canal, and due to the absence of a specific carrier of glutathione at the level of cell membrane. Because direct supplementation of glutathione is not always successful, supply of the raw nutritional materials used to generate GSH, such as cysteine and glycine, may be more effective at increasing glutathione levels. Other antioxidants such as ascorbic acid may also work synergistically with glutathione, preventing depletion of either. Additionally, compounds such as N-acetyl-cysteine (NAC) is important in helping regenerate glutathione levels. Low glutathione is commonly observed in wasting and negative nitrogen balance, as seen in cancer. These effects are hypothesized to be influenced by the higher glycolytic activity associated with cachexia, which result from reduced levels of oxidative phosphorylation. Therefore; we recommend supplementation with NAC in 1200 mg/day to be effective in cancer prevention.

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