Evaluation of the Radioprotective Effect of Green Tea Extract (GTE) in Gamma-Irradiated Mice

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CONTENTS

INTRODUCTION

Radiation Therapy

People have been exposed to radiation through the millennium, but it is only in the last approximately 100 years that the potential diagnostic and therapeutic use of radiation has been harnessed. This beneficial use was launched by the experiments of Wilhelm Roentgen, who in 1895 found that x-rays could pass through materials that were impenetrable to light. Roentgen could be considered the father of diagnostic imaging for demonstrating that x-rays could be used to produce a photographic image of the dense bony structure of the human hand. Emil Grubbe provided one of the early examples of the therapeutic use of radiation by treating an advanced ulcerated breast cancer with x-rays in January 1896 [(1)].

The role of radiation therapy (RT) in the treatment of cancer and noncancerous conditions has expanded dramatically in recent years and both the fields of radiation oncology and the technology used for treatment have seen many advances. Based on a greater understanding of radiobiology and physics, patients can now receive radiation as a primary treatment modality. Radiation therapy may be used alone or in combination with chemotherapy [(2)], surgery [(3)] or biotherapy [(4)]. Collaboration of the multidisciplinary team is critical to ensure optimal patient outcomes.

Radiotherapy is the art of using ionizing radiation to destroy malignant tumors while minimizing damage to normal tissue [(5)]. The aim of the therapy is to deliver a precisely measured dose of irradiation to a defined tumor volume with as little damage as possible to surrounding healthy tissue, resulting in irradiation of tumor, high quality of life and prolongation of survival at competitive dose [(6)]. Although higher doses of radiation can produce better tumor control, the dosage that can be given is limited by the possibility of normal tissue damage. Ulceration, fistulas, severe fibrosis, and strictures may develop months or years after treatment, severely affecting the quality of life [(7)].

Goals of Treatment Approaches:

Radiation plays a major role in the treatment of patients diagnosed with cancer. Approximately 60 % of cancer patients will receive radiation at some point in their disease trajectory, either to cure, control, or palliate the disease [(6)].

If the tumor is diagnosed at an early stage, cure is possible. Patients undergoing a curative course of radiotherapy often face vigorous and lengthy treatment. In such cases, the total dose of radiation may be higher and toxicities of treatment may be more sever. Patients with early-stage Hodgkin's disease [(8)], skin cancer [(9)] and carcinoma of the cervix [(10)] are often treated with radiation alone.

For certain types of cancer and those in later stages cure or eradication is not possible. In such cases, control of the cancer with radiation therapy for periods ranging from months to years may be the goal. Recurrent breast cancer, some soft-tissue sarcomas and lung cancer are controlled by radiation therapy in combination with surgery [(11)].

Palliation may be another goal of radiation therapy. Relief of pain, prevention of pathological fractures and return of mobility can be achieved with radiation to metastatic bone lesions for primary sites such as breast, lung and prostate. Pain relief often is dramatic, and it is not uncommon for one individual to receive multiple palliative courses to different bony structures over the course of several years. Radiation therapy contributes significantly to improved quality of life for the person with bone metastases. Palliative radiation therapy also is given for the relief of central nervous system (CNS) symptoms caused by brain metastasis or spinal cord compression [(12)].

Types of Radiation Damage:

The effects of radiation are characterized by the survival time of the species and various stages of syndromes that develop following total-body irradiation. Different tissues of the body respond differently to radiation, due to varying degrees of radiosensitivity. The radiosensitivity of a given species is commonly characterized by the lethal dose, $LD_{50/60}$, which is the dose that kills 50 % of the species in 60 days. The $LD_{50/60}$ for humans is 400 to 600 rad (4 to 6 Gy); dogs, 300 rad (3 Gy); and for mice, 900 rad (9 Gy) [(13)].

The typical side effects of radiotherapy can be subdivided into early and late depending on when they appear in relation to treatment. When an adult subject is irradiated over the entire body, various syndromes are manifested depending on the dose applied [(13)].

A) Acute Radiation Syndromes:

Acute radiation syndromes appear in four stages: prodromal, latent, manifest illness, and recovery or death. Each stage is dose dependent and can last for a few minutes to weeks. A minimum of 200 to 300 rad (2 to 3 Gy) is required for all four stages to be seen and can cause death [(14)].

In the prodromal stage, major symptoms are nausea, diarrhea, and vomiting. In the latent stage, biological damage slowly builds up without manifestation of any syndromes, again lasting for hours to weeks, depending on the dose. During the manifest illness stage, radiation syndromes appear as a result of the damage to the organs involved after the latent period, and the subject becomes ill. In the last stage, the subject either recovers or dies [(12)].

There are three categories of syndromes in the manifest illness stage depending on the dose: hemopoietic or bone marrow, gastrointestinal (GI), and cerebrovascular. These subcategories reflect both the relative sensitivities of various organ systems to radiation and the time required to produce effects on the overall health of the organism [(15)].

i- Hematopoietic Syndrome

Hematopoietic syndrome appears at a total body dose of 250 to 500 rad (2.5 to 5 Gy) following irradiation. At this dose, the stem cells of the hematopoietic system, precursors of mature red blood cells (RBCs) and white blood cells (WBCs) are greatly affected, so much so that they lose the ability to reproduce. Also, the number of lymphocytes is greatly depressed, whereby the immune system of the body is suppressed. Loss of blood cell counts can be noticed at a dose as low as 10 to 15 rad (0.1 to 0.15 Gy). Thus the body loses the defense against bacterial and viral infection and becomes susceptible to them. Immunosuppressant by radiation occurs at doses as low as 100 rad (1 Gy) and 90% to 95% of immunosuppression can take place in humans at doses of 200 to 400 rad (2 to 4 Gy) [(16)]

At this dose level, the platelet count is drastically reduced, and therefore bleeding gradually progresses. Fever, bleeding, and infection result, followed by ultimate death in 10 to 21 days. Whereas at does <100 rad (1 Gy) survival is almost certain, survival is virtually impossible at does >500 rad (5 Gy) [(17)].

ii- Gastrointestinal Syndrome

Gastrointestinal (GI) syndromes are expressed at a total body dose of 500 to 1000 rad (5 to 10 Gy) and include prodromol syndromes such as nausea, vomiting and diarrhea of more severity that appears few hours after exposure. The primary effect of radiation exposure in this range is that the intestinal crypt cells are destroyed and not replaced, and consequently the mucosal lining (villi) shrinks and hardens whereby the gut becomes nonfunctional. Because of the denudation of the gut, an intestinal ulcer may develop. These GI syndromes are also accompanied by drastic hemopoietic syndromes including immunosuppression, loss of white blood cells, and infection [(18)].

Thus, the loss of nutrients through ulcers, in combination with bacterial infection and excessive bleeding, results in GI death in 3 to 10 days after radiation exposure. Only aggressive medical treatment in the early stages of exposure may lead to recovery in cases at the lower end of the dose spectrum [(15)].

iii- Cerebrovascular Syndrome

For radiation doses above 100 Gy, the majority may die within 48 hours as the result of the central nervous system syndrome. The symptoms are irritability and hyperactive responses (almost like epileptic attacks) which are followed rapidly by fatigue, vomiting and diarrhea. The ability to coordinate motion is lost and shivering occurs followed by coma. Then respiratory problems occur which eventually lead to death [(19)].

The symptoms described are due to damage to the brain, nerve cells and blood vessels. Immediately, permeability changes take place in the blood vessels resulting in changes in the electrolyte balance. The loss of liquid from the blood vessels leads to increased pressure in the brain. It is possible that the respiration center in the brain is particularly damaged. Death may occur in a few days or, at higher doses (greater than 100 Gy), within hours. Because of the involvement of brain and nervous system tissues, the cerebrovascular syndrome (CVS) is

sometimes referred to as central nervous system syndrome. The hematopoietic and GI systems are also severely damaged at this dose level but would not contribute to the cause of death in such a short time [20].

B- Late Radiation Effects

The long-term or late effects of radiation cause various syndromes long after the radiation exposure. These may appear after acute radiation syndromes subside following exposure to a single large dose or after exposure to many smaller doses over a period. The late effects may be somatic or genetic, depending on the respective cells involved. Somatic effects are seen in the form of carcinogenesis, life-shortening, and embryologic damage. On the other hand, genetic effects result in abnormalities in the offspring [16].

Cellular Response to Radiation:

The biological effects of radiation on humans are the result of a sequence of events that follows the absorption of energy from ionizing radiation and the organism's attempts to assault. The effects of radiation takes place at cellular level, with consequences developing in the tissues, organs and/or the entire body depending on the treatment site. Radiation's biological influences on these cells are mediated by variety of biochemical, genetic, and kinetic factors. They are also affected by the dose, type, and rate at which radiation is given [21].

When photons or particles interact with biological material they result in ionizations that can either directly interact with subcellular structures or they can interact with water, the major constituent of cells, and generate free radicals that can then interact with subcellular structures (figure 1) [22].

A- Direct Action

The direct effects of radiation are the consequence of the DNA in chromosomes absorbing energy that leads to ionizations. This is the major mechanism of DNA damage induced by protons and neutrons and is termed high linear energy transfer (Fig. 1). Ionizing radiation causes base damage, single-strand breaks, double-strand breaks, and sugar damage, as well as DNA-DNA, and DNA-protein cross links. The critical target for ionizing radiation-induced cell inactivation and cell killing is the DNA double-strand break. Such unrepaired breaks or alterations in a base lead to mutations that result in impaired cellular function or cell death [23, 24] .

B- Indirect Action

In contrast to the direct effects, indirect effects of ionizing radiation result from the interaction of photons with other molecules in the medium such as water results in the production of free radicals. Free radicals are usually denoted by a dot (•) placed to the left or right of the chemical symbol depending on the position of the unpaired electron. Most of the energy deposited in cells is absorbed initially in water (the cell contains 70 %–80% water),

leading to the rapid (within 10^{-5}–10^{-4} s) production of oxidizing and reducing reactive radical intermediates (the OH radical [•OH], an oxidizing agent, is probably the most damaging), which in turn can react with other molecules in the cell. [25] Some of theses free radicals possess a lifetime long enough to be able to diffuse to the nucleus and interact with DNA in the chromosomes. This is the major mechanism of DNA damage induced by x-rays and γ rays and it has been termed low linear energy transfer [26].

These free radicals can interact with critical macromolecules, such as DNA, proteins or membranes, and can induce cell damage and, potentially, cell dysfunction and death. Damage to DNA may be the most important factor in cell death [27].

It's generally agreed that a direct hit (.i.e. DNA damage and chromosomal aberrations) accounts for the most effective and lethal injury produced by ionizing radiation. However because of the relative ratio of water to DNA in a single cell, the probability of indirect damage through ionization of intracellular water is much greater than the probability of damage from direct hit [28].

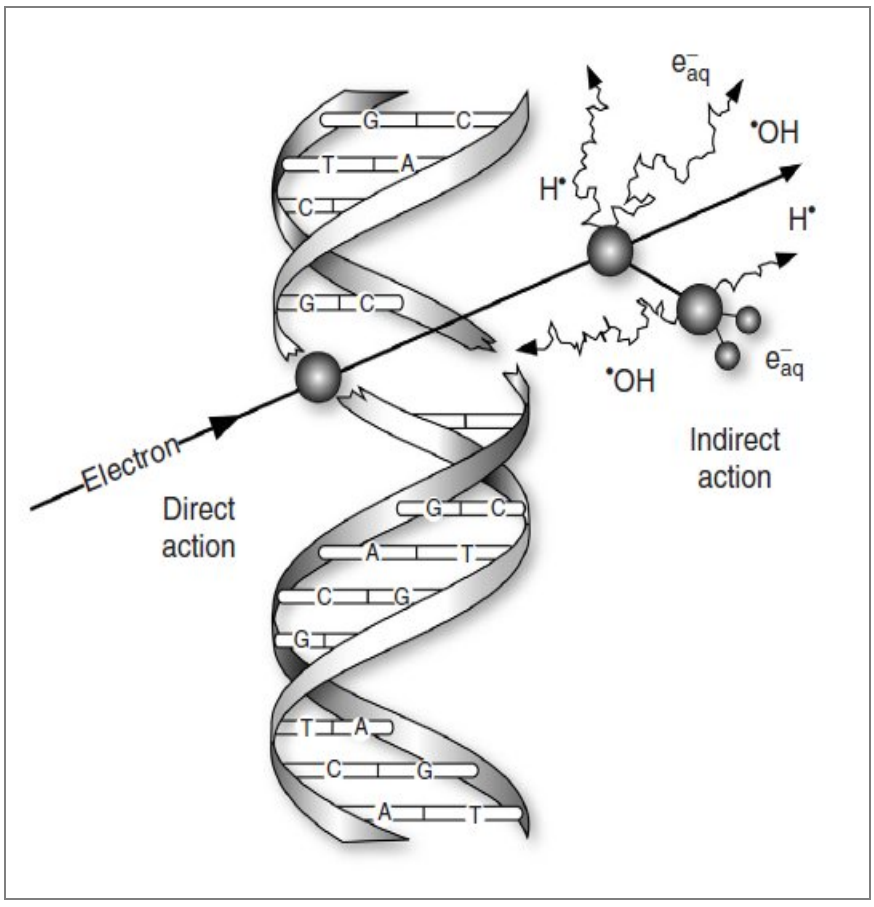

Figure (1): Direct and indirect actions of radiation. [24]

The Effect of Radiation on Cell Survival

The cellular responses to various form of radiation, including ionizing- and UV-irradiation or exposure to electromagnetic fields is manifested as irreversible and reversible structural and functional changes to cells and cell organelles. [29] The morphological changes observed in hepatocytes post irradiation are collectively the characteristic alterations in cells undergoing apoptosis. Apoptosis is expressed as an active, intrinsic mechanism based on the concerted action of specific proteases (caspases) and endonucleases. [30] Many observations suggest that apoptosis is the main form of ionizing radiation-induced cell death in many types of cells [31, 32].

The antitumor activity of radiotherapy is to a degree dependent on the induction of tumor cell apoptosis in response to oxidative stress and oxygen radical induced DNA damage. The process of apoptosis starts within minutes following irradiation and lasts for several hours. However, time- and cell position-dependent variations in the apoptotic response have been observed in several tissues. [31] Some types of cells such as cultured fibroblasts (V79, L-929), Chinese hamster ovary (CHO) cells, as well as many human tumor cell lines, appear in practice unable to undergo apoptosis in vitro and they generally die by necrosis. The dose of irradiation may play a role in determining the type of cell death, high doses may cause cell destruction by necrosis in lymphoid cells, while lower doses induce apoptosis [33].

Shrinkage and fragmentation of nuclei are the main morphologic features of cells dying by apoptosis. Condensation and clumping of chromatin, redistribution of nuclear pores, and dissolution of nuclear laminae accompanies this process. The chromatin fragments may appear scattered in the cytoplasm in the form of so called micronuclei. The cell as a whole shrinks and finally breaks up into fragments which become engulfed by neighboring cells [34, 35].

Alterations in mitochondrial structure (swelling and disappearance of cristae) and function (drop in mitochondrial transmembrane potential) occur in early stages of apoptosis and may precede and/or accompany nuclear changes. [36] Disintegration of Golgi complex and dilatation of endoplasmic reticulum as well as disappearance of microvilli [37] and cell contacts [38] and formation of blebs [39] are commonly observed in apoptotic cells.

Free Radicals and Oxidative Stress

Oxygen free radicals, more generally, reactive oxygen species (ROS) as well as reactive nitrogen species (RNS) are products of normal cellular metabolism. ROS are either free radicals, reactive anions containing oxygen atoms or molecules containing oxygen atoms that can either produce free radicals or are chemically activated by them [(40)]. Examples are hydroxyl radical, superoxide, hydrogen peroxide, and peroxynitrite. ROS and RNS: (i) are generated during irradiation by UV light, by X-rays and by gamma rays; (ii) are products of metal-catalyzed reactions; (iii) are present as pollutants in the atmosphere; (iv) are produced by neutrophils and macrophages during inflammation; (v) are by-products of mitochondria-catalyzed electron transport reactions and other mechanisms [(41)].

ROS/RNS are known to play a dual role in biological systems, since they can be either harmful or beneficial to living systems. Beneficial effects of ROS involve physiological roles in cellular responses to anoxia, as for example in defense against infectious agents and in the function of a number of cellular signaling systems. One further beneficial example of ROS at low concentrations is the induction of a mitogenic response. In contrast, at high concentrations, ROS can be important mediators of damage to cell structures, including lipids and membranes, proteins and nucleic acids [(40)]. The delicate balance between beneficial and harmful effects of free radicals is a very important aspect for living organisms and is achieved by mechanisms called "redox regulation" [(42)].

Oxidative stress and nitrosative stress are the main causes of the potential damage of free radicals in biological systems [(43)]. Oxidative stress represents a disturbance in the equilibrium status of prooxidant/antioxidant reactions in living organisms. It may be a result of an increase in oxidant generation, a decrease in antioxidant production or a failure to repair oxidative damage [(44, 45)].

Reactive oxygen species:

1. Superoxide anion ($O_2^{\bullet -}$)

The addition of one electron to dioxygen will form the superoxide anion radical ($O_2^{\bullet -}$). Superoxide anion arises either through metabolic process or following oxygen "activation" by physical irradiation. It is considered the primary ROS, and can further interact with other molecules to generate "secondary" ROS either directly or prevalently through enzyme- or metal-catalyzed processes [(46)]. The production of superoxide occurs mostly within the mitochondria of a cell [(47)]. The mitochondrial electron transport chain is the main source of ATP in the mammalian cell and thus it is essential for life. During energy transduction, a small number of electrons "leak" to oxygen prematurely, forming the oxygen free radical superoxide instead of contributing to the reduction of oxygen to water. Superoxide anion has been implicated in the pathophysiology of a variety of diseases [(48)].

2. Hydroxyl radical ($^{\bullet}OH$)

The hydroxyl radical $^{\bullet}OH$ is the neutral form of the hydroxide ion. The hydroxyl radical has a high reactivity making it very dangerous radical with a very short half life in vivo [49]. Thus when produced in vivo $^{\bullet}OH$ reacts close to its site of formation.

It can be generated through a variety of mechanisms. Ionizing radiation causes decomposition of H_2O resulting in the formation of $^{\bullet}OH$ and hydrogen atoms. $^{\bullet}OH$ is also generated by photolytic decomposition of alkylhydroperoxides. Production of $^{\bullet}OH$ close to DNA could lead to this radical reacting with DNA bases or the deoxyribosyl backbone of DNA to produce damaged bases or strand breaks [50].

3. Peroxyl radical ($ROO^{\bullet}$)

Peroxyl radicals ($ROO^{\bullet}$) are additional reactive radicals derived from oxygen that can be formed in living systems are. The simplest peroxyl radical is the dioxyl (hydroperoxyl) radical $HOO^{\bullet}$ which is the conjugate acid of superoxide $O_2^{\bullet-}$ [51].

The chemistry of this type of molecule varies according to the nature of the R group, the local environment, and the concentration of oxygen and of other reactants. Perhaps the most interesting feature of peroxyl radicals is the diversity of those biological reactions in which they participate. The detection and measurement of lipid peroxidation is most frequently cited as evidence to support the involvement of peroxyl radical reactions in human disease and toxicology. Peroxyl radicals are involved in DNA cleavage and protein backbone modifications. Peroxyl radicals synergistically enhance the induction of DNA by superoxide [52].

4. Hydrogen peroxide (H_2O_2)

Oxygen consumption in the peroxisome leads to H_2O_2 production, which is then used to oxidize a variety of molecules. When peroxisomes are damaged, H_2O_2 releases into the cytosol which is significantly contributing to oxidative stress [53]. It is also generated in biological systems through the dismutation of superoxide anion. This dismutation occurs spontaneously or can be catalyzed by the enzyme superoxide dismutase [54].

H_2O_2 is able to cross cell membranes and it can easily diffuse between living cells. H_2O_2 then can -although being only a weak oxidant- inactivate some enzymes via oxidation of essential thiol (-SH) groups [55]. For example exposure of cells to large amounts of H_2O_2 can lead to adenosine triphosphate (ATP) depletion by inhibition of enzymes responsible for glycolysis [56].

Reactive Nitrogen Species (RNS):

Nitric oxide radical ($NO^{\bullet}$) is a small molecule that contains one unpaired electron on the antibonding orbital and is therefore a radical. $NO^{\bullet}$ is generated in biological tissues by specific nitric oxide synthases (NOSs), which metabolise arginine to citrulline with the formation of $NO^{\bullet}$ via a five electron oxidative reaction [57].

Over production of nitrogen species is called nitrosative stress. This may occur when the generation of reactive nitrogen species in system exceeds system's ability to neutralize and eliminate them. Nitrosative stress may lead to nitrosylation reactions that can alter the structure of proteins and so inhibit their normal function.

The toxicity of $NO^{\bullet}$ is predominantly linked to its ability to combine with superoxide anions producing the much more oxidatively active molecule, peroxynitrite anion ($ONOO^-$).

This reaction usually occurs during the oxidative burst triggered during inflammatory processes. $ONOO^-$ is a potent oxidizing agent that can cause DNA fragmentation and lipid oxidation [58].

$$NO^{\bullet} + O_2^{\bullet -} \rightarrow ONOO^-$$

Oxidative Damage:

Oxidative Damage to DNA:

DNA is considered a major target of reactive oxygen species attack. Permanent modification of genetic material resulting from these "oxidative damage" incidents represents the first step involved in mutagenesis, carcinogenesis and ageing. In fact, as is well established, free radical-mediated DNA damage has occurred in various cancer tissues. To date, more than 100 products have been identified from the oxidation of DNA [59].

The vast majority of DNA damage affects the primary structure of the double helix by chemically modifying the bases themselves. These modifications can in turn disrupt the molecule's regular helical structure by introducing non-native chemical bonds or bulky adducts that do not fit in the standard double helix [60].

The main types of chemical damage that arise mainly from endogenous processes that generate oxygen radicals, especially superoxide and peroxides are:

1. Oxidation of DNA bases; guanine is the most easily oxidized among the four DNA bases, because the oxidation potential of guanine is lower than the other bases, adenine, cytosine and thymine [61, 62].
2. Alkylation of bases (usually methylation), such as formation of 7-methylguanine.

3. Hydrolysis of bases, such as depurination and depyrimidination.
4. Mismatch of bases, due to DNA replication in which the wrong DNA base is stitched into place of a newly forming DNA strand [63].

In addition to ROS, reactive nitrogen species (RNS) such as peroxinitrites and nitrogen oxides have also been implicated in DNA damage. Upon reaction with guanine, peroxynitrite has been shown to form 8-nitro-guanine. Due to its structure, this adduct has the potential to induce G:C→T:A transversions. While the stability of this lesion in DNA is low, in RNA, however this nitrogen adduct is stable [64].

One of the most abundant and easily measured products of DNA oxidation is 8-oxo-7, 8 dihydro-2′-deoxyguanosine (8-oxo-G) (figure 3).

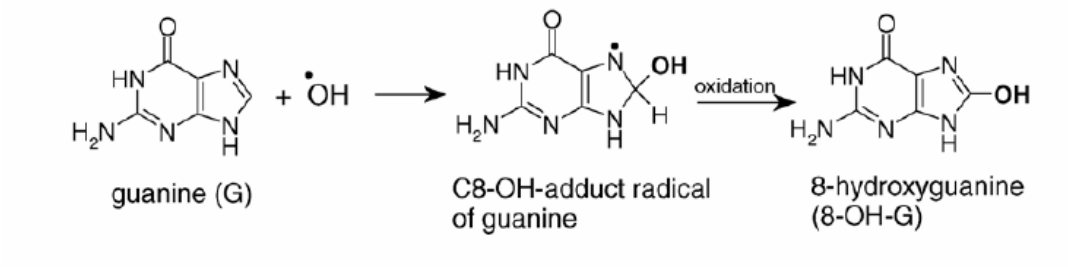

Figure (2): Reaction of guanine with hydroxyl radical

8-oxo-G is considered to be a mutagenic DNA lesion. It was reported that misincorporation of adenine occurs opposite 8-oxo-G during DNA synthesis, leading to G→T transversion [65-67].

Oxidative damage to DNA is continuously ongoing but is also repaired with high efficiency in the cell in the body [68, 69]. When damaged DNA is repaired, 8-oxo-G produced is excreted in urine without further metabolism [70, 71]. 8-oxo-G has been widely used as a sensitive marker of oxidative DNA damage and of the total systemic oxidative stress in vivo. It is also a potential biomarker of carcinogenesis [72, 73].

Oxidative Damage to Proteins:

Exposure of proteins to ROS results in dramatic changes in their structure, stability and function. Oxidative attack on proteins results in site specific amino acid modification, fragmentation of the peptide chain, aggregation of cross-linked reaction products, altered electrical charge and increased susceptibility to proteolysis [74, 75]. The amino acids in peptide differ in their susceptibility to attack, and the various forms of activated oxygen differ in their potential reactivity. Primary, secondary and tertiary protein structures alter the relative susceptibility of certain amino acids. Generally, sulfur containing amino acids, and thiol groups specially, are very susceptible sites of oxidative attack. Activated oxygen can abstract H atom from cysteine residues to form