Review Article

Sources of Ionizing Radiation and their Biological Effects: An Interdisciplinary View, from the Physics to Cell and Molecular Biology

Abstract

Exposure to the IR is common to certain people like professionals handling radioactive materials or to the patients undergoing radio-diagnostics and radiotherapy or as millions of people who travel by air are exposed by X-rays scanning every day. Though it is indirect cause, IR may trigger mutation in healthy cells which further induces molecular alterations. It´s known that ionizing radiation generates free radicals from cytoplasmic water and ultimately induces biomolecules lesions such as DNA damage. These damages may lead to neoplasm in normal and healthy cells however IR is not by itself a recognized and indisputable carcinogen present in the environment. In order to develop some type of cancer, they have to interact within the organisms and cells with other multiple factors of high complexity from physiological to environmental components (genetics of the living being, cellular microenvironment, epigenetic factors, environmental conditions, and others, perhaps still unknown). Here we discuss and present IR effect on living cells, ways of damage determination and compounds reported as radioprotectors.

Keywords: *Comet assay, effects and origin of cancer, ionizing radiation sources, radiobiology, radioprotectors and radiomitigant agents*

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Introduction

Ionizing radiation (IR) sources today imply a well-recognized physical risk for living beings from all ranges of exposure. IR implementation in health services was presented as the cause and effect and as an originating factor of nonspecific lesions and various types of cancers to those within a proximal range. It's well known the cases of cancer in pioneering medical radiologist from the late 19th and early 20th century which are first evidences of IRs' exposure as carcinogenic hazard. From these first evidences, the bases of radiobiology were very quickly established by Bergonié and Tribondeau.[1-4]

The sources of IR within various criteria can be classified as natural and artificial, where all are characterized by being able to generate emissions of particles (with or without net charges, positive or negative), photons, or electromagnetic radiation (nonmaterial or photon emissions), which carries sufficient amount of energy to produce reversible or not cleavage of bonds between atoms, this cause the formation of stable or nonstable molecules that in turn make up the inert and living matter. The natural sources of IRs are found around our planet, and the rest come from the universe. One example is the sun, where nuclear fusion reactions take place to generate light and heat. On the other hand, on our planet, some heavier elements such as uranium and thorium series emit IRs to other atomic species in the so-called radioactive chains. Together, all these natural sources originate from what is known as the natural background (NB) of IR, which can be evaluated with the corresponding units of measure.[4]

Sources of ionizing radiations and their interaction with matter and corresponding units

The consequence of the interaction between different types of IRs, beams, and matter is that they cause the formation of ions, free radicals, or chemical radicals. Any IR source can generate a nuclear field around it and give rise to the terms: events or emissions per unit of time which have the Becquerelium – Bq – as an International System Unit (1 Bq = 1 event/second). Historically, before Bq, Curie unit was used as an activity unit, especially for natural and artificial material sources.[5]

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In the case of artificial sources, such as X-rays, another concept is specifically defined called the exposure rate ($\dot{X} = X/\Delta t$), whose units are expressed in Roentgen (R) by the unit of time. It is not an international unit, but it is still used in hospital practice in South America and around the world. The exposure rate is briefly the amount of IR in the proximity of a target or person, and it is described by milliroentgen per hour – mR/h –. This amount produces a biological effect on the body exposed to the IR, and it is described or measured by nanosievert per hour – nSv/h – the dose rate. For example, an X-ray that has a radiological dose rate of 1 mR/h would be roughly equal to an exposure rate of 10,000 nSv/h.

At a defined exposure rate, if you know the amount of time (Δt) you were emitting, you can know the total exposure that occurs around it (in this case, it is generally air from the environment to the environmental conditions of the equipment), at a constant distance from the origin, that is $X = \dot{X}. \Delta t$. The exposure unit R in the old unit system is still used around the world and is the unit of measurement that describes the number of ion pairs produced in air.[4]

Briefly, the above description means that the IR sources produce and emit a certain number of radioactive events to their surrounding space, per unit of time, with a given amount of energy, and then they are transferred through space to interact with a material entity. Our interest is to study the effects when it comes to living beings and establish an animal model to study its effects *in vivo*. Living beings are constituted by elementary units called cells, in cells water is the most abundant molecule accounting for more than 70% of total cell mass. Consequently, the interactions between IR and water than other constituents of cells is of central importance. IRs do not have a preferred target, but in living beings, the DNA after IR effect on water is the most vulnerable target.[5]

Ionizing radiations and the mechanisms of their effects at different levels

IR observable effects on living beings such as measurable deleterious effects on cells, increasing reactive oxygen species (ROS), generation of single-stranded DNA breaks and double-stranded DNA breaks (DSBs) are currently known to depend on the number of events originated in a source (the energy emitted); the number of interactions with the target material; and the energy, mass, and net charge of the emitted radiation beams during their trajectory through the target material; this is defined as linear energy transfer which is conceptually expressed as energy loss per unit length of the trajectory of the incident beam, and is usually stated in Joule/μm. This amount in relation to biochemical or biology data can give an idea of the amount of damage caused in the structural units of living beings, the capacity of the enzymatic systems to repair them, and whether they are saturated or not in their action.[3-5]

The energy in which the incident beam (E_i) enters the matter suffers a progressive decrease during its trajectory due to a large number of small shocks (which reduce its E_k in the path) by the interactions with the material medium, together with other occasional interactions where other electrons can be ejected if they receive enough energy (threshold) to produce secondary electrons or δelectrons (direct and indirect actions of radiation) [Figure 1 modified from Hall and Giaccia, 2012].[4]

The observable effects can also be classified into two groups, taking into account the characteristics of the damage they cause. These are the deterministic and stochastic (random) effects. For the deterministic effects, by empirical evidences that have been accumulated, we know in advance what effects they will produce in a living being, from the appearance of a simple lesion or even death, depending the net dose value absorbed by the living system. This means that for certain quantities of doses absorbed by this and from its threshold value, it can be predicted what will happen in a time range known *a posteriori* of the event. The deterministic effect usually occurs at high doses and high dosage rates of an IR source, as in the case of radiotherapy or treatment with high activity sources in patients.[2-4] On the other hand, there are sources of IR that emit relatively very low dose rate as the NB, which gives rise to the stochastic or random effects. The major difference between any inert matter and a living being is that the latter can repair the injuries or damage produced by the IR within a certain range, or at least mitigate its action, because the repair is an enzymatic and chemical system. It has some degree of saturation that upon reaching the IR gives a measurable biological effect[6] [Figure 2].

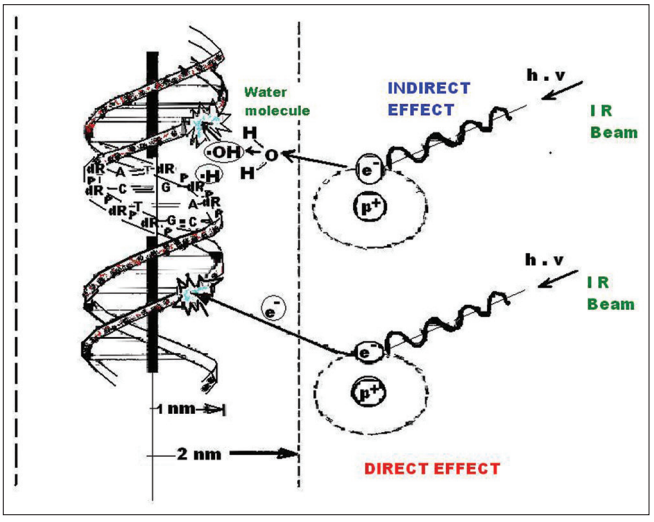

Figure 1: Scheme that represents (above) the indirect damage, by adding a radical •OH to a nitrogenous base of a DNA strand. And below, the direct damage by the action of a photon or an electron torn from the surrounding material and impacting the DNA like a projectile, breaking the covalent bonds of a strand. Adapted from Giaccia and Cols

Different effects produced at the cellular and molecular level

The lesions and damages within cellular and biomolecular structures can be measured, and it depends on the relative radiosensitivity of these matters.[1] As per target, the majority of the biomolecules can be included for damage because IR does not have any preferential target. In that case, biological systems can repair the damage or can replace them by new ones, incorporating them from the media or by new synthesis using the genetic machinery if the DNA is damaged in some way; this may generate some type of genomic instability (which can range from missing parts of chromosomes, irreversible links, to mutations). The information error generated by DNA damage can interfere with downstream or upstream processes in the targeted cell and its surroundings (bystander effects), generating changes in the expression of cell cycle checkpoint pathways, DNA replication, cell signaling, and pathway expressions.[7,8,2] The ionizing radiation-induced bystander effect (RIBE) is broadly defined as the occurrence of biological effects in nonirradiated cells as a result of exposure of other cells in the population to radiation. RIBE shows nonlinear dose response; it is more pronounced at low doses of radiation and tends to disappear, though not always, at high radiation doses, suggesting an on–off mechanism. As a result, it is frequently linked to "low dose" radiation effects and thus to radiation system protection.[9] One of the early respondents to IR damage is the mechanism initiated by the expression of the p-53 gene that triggers cell death and apoptosis. Paradoxically, the same IR can affect p-53-associated pathways, causing an opposite effect as per p-53 gene expression, or the inability to die that makes a group of cell types to perpetuate over time and grow in numbers within the body. This is a characteristic shared by many types of cancer, and that is why IRs are a recognized carcinogen, even at "low" doses.[6,10] The damage caused may be reversible to some extent or irreversible, or deleted by complete elimination of the cell damaged, by necrosis or apoptosis. In brief, the responses to the damage in living cells can range from the complete repair of the damage to programmed cell death. The problem between these two extremities of the long-term effects of IR and low dose rates, usually related to stochastic effects, is the propagation of a genetically altered cell (clonal effect) which can sometimes generate a clonally altered population and a cancer cell type which may be in quiescent state for long time.[11,12]

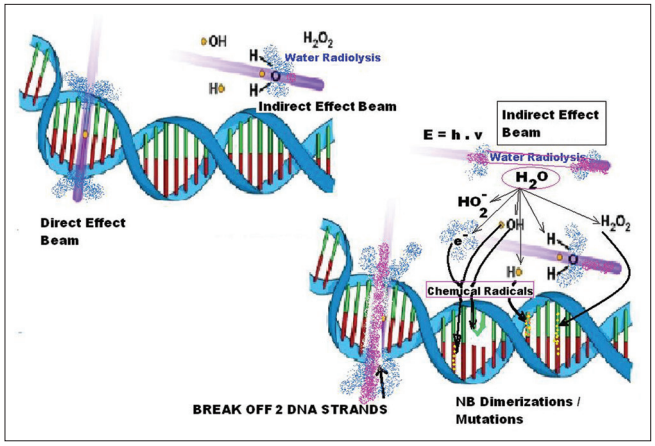

Figure 2: Ionizing radiation effects on DNA

The capacity of the cells and organisms to repair injuries of their functional and structural molecules allows them to preserve their genomic integrity; this is required for a proper organ and cell functioning mechanism. DNA repair systems are efficient rectifiers of their genetic errors, which are phylogenetically conserved from bacteria and fungi to mammals. These mechanisms are very complex and are very much studied. However, these are yet not fully clarified because they also interact with other associated systems such as metabolic networks, cell cycles, and proliferation (most of them which are activated by the interaction with IR and promote cell survival while maintaining their genetic integrity).[11,13,12] When the damage is not repaired or is partially repaired, the cell manages to continue and may even progress to clonal replication, and as an altered cell, this would generate what is known as a sublethal damage, giving rise to the progenies or populations of dysfunctional cells. Some sublethal damages can be measured by the appearance of a micronucleus, and its visualization can be done by techniques described early by Fenech,[14] Kissling *et al*.,[15] and Witt *et al*.[10] The immune system also plays a critical role in eliminating this type of "abnormal" cells, which can occur in certain states of immunosuppression. The most radiosensitive cells are mature lymphocytes, which are key components for specific cellular and humoral immunity. Nevertheless, some authors have reported contradictory results such as immunosuppression, normality, or greater immunocompetence at low doses of photonic IR.[16,17,12] Another way to measure the grade of damage with high-to-medium doses rates is the measure of leukocyte formula (UNSCEAR). However, the results vary from each individual, with sources of relatively low dose rates evidencing intrinsic differences in DNA repair or immune systems.[12]

Regarding X-ray service environment and risk for stochastic or deterministic effect, we consider dose rates or "low" exposure rates to those that are a few times higher than the order of magnitude of the natural background, which may be the case for scattered IR bundles around the environment of X-ray services such as hospitals, dental clinics, and inside the facilities near their patients; these low exposure rates can also interact with health-care professionals and the public if there are no measures for isolation.

Perhaps, other activities or circumstances to low exposure rate sources are pilots and respective flight attendants,

astronauts, customs security personnel at airport screenings by X-ray, and many customs areas nowadays. Therefore, all these attenuated photons of relatively low energy are potentially responsible for causing stochastic effects, which although have the characteristic of causing damage with the possibility of a very small quantity, but the risk of causing some damage is never equal to zero.[2,18] We have been able to measure a statistically significant damage in a Balb/c male mice model caused by low doses closest to the natural background and no more than can be received by X Ray operator personel (Martinez Marignac, V and Favant, J. L, In Press process, 2019). In this regard, the concept of cause/effect of the IR still presents a controversy about which one is the mathematical model that would explain the different experimental aspects observed. Nevertheless, it is known that IR produces DNA damage.

Consequently, different ways of observing a given effect on studied cell populations were established as the doses received by them were increased or decreased. With regard to clinical and scientific investigations of IR effects on living beings, for a long time in the scientific methodology, we use experimental animal models, and side by side isolated culture cells.[12,6] The animal or cell culture models that have been used historically range from simple cells, prokaryotes (bacteria such as *Micrococcus* spp.), eukaryotes (yeast or unicellular fungi, *Saccharomyces* spp., and others), to animal cells of different genus and species, such as rats and albino mice (*Mus* spp., *Rattus norvegicus,* and others).[2,5,19]

In addition, although there are physiological similarities between mammals, they are not totally extrapolated to humans, but there are some approximations that are performed experimentally and as preclinical studies.[2,4]

The validation of a model is a step of ethical and financial implications, which pursuits the establishment of a robust reference of good predictive value and allows to define the application domain.[12,17,20,21]

Exogenous sources of free radicals in the cellular medium and their effects

IR is not only by itself a recognized and indisputable carcinogen potentially present in the environment. In order to develop some types of cancer, they have to interact within the organisms and cells with other multiple factors of high complexity from physiological to environmental components (genetics of the living being, cellular microenvironment, epigenetic factors, environmental conditions, and others, perhaps still unknown).[4] When IRs interact with living cells, almost instantaneously physical interactions take place, known as Rayleigh scattering, photoelectric, and the Compton effect, which are of relatively low energies. This stage constitutes the early effects or direct effects which produce reactive groups that can then break bonds between atoms. When IRs interact with living cells there are also what it is called late effects. As previously discussed, one of the component in high proportion is water molecules, whose interaction with the IR generates its own radiolysis that then exerts indirect actions, which are example for the denominated late effects, producing chemical and biological changes, alterations or oxidation damaging biomolecules such as on lipids, carbohydrates, proteins, or nucleic acids (NA), and this is produced without preferences, no more than the distance of the interactions carried about. Consequence, one of the indirect mechanisms of action of the IR on the cells is to produce free radicals and agents or ROS. ROS are free radicals, reactive radicals, simply radical atoms, or groups of atoms that have one or more unpaired electrons, like O_2^- among others, and are able to react with other molecules in different ways. This happens from donating an unpaired electron to another molecule or snatch an electron in order to reach a stable state[12] in cells. This has a direct effect on their metabolic homeostasis rather than a more direct effect on DNA.[2,22]

In all these reactions, a free radical transforms another molecule or atom into another radical, generating a chain process where only in the case where two radicals interact, the progressive effect comes to an end. One example is water, which, in some cells, composes up to 80% of the intracellular mass and creates generic radicals derived from its molecules and dissolved O_2 in the cellular environment. These substances are very reactive (oxidants) and are capable of causing alterations or additions in other neighboring molecules by joining them into an alkylation reaction. This reaction causes mutations when there are interactions with DNA sequences, enzyme activity alterations, and gene expression when the interactions are with RNA or proteins. This impact of ROS results in cellular aging, damages of its biomolecular components, cancer initiation, and other associated pathologies. Nevertheless, ROS are not only produced or increased by external factors such as IR sources, but they are also the result of cellular metabolism, mitochondrial respiration and the maintenance of cellular homeostasis.[23] Subsequent to radiation exposure, mitochondrial DNA (mtDNA) might be preferentially damaged or lost due to oxidative stress with an ensuing decrease in respiratory chain activity and decrease of mitochondrial function.[24]

Although there are already known endogenous protective mechanisms (i.e., superoxide dismutases [SOD]), there are also exogenous substances that can be incorporated in the diet or used as drugs that will work as a radioprotective agent or a radiomitigant. Many of these molecules have being studied in mouse models, such as the strain of SCID or BALB/c, which show a greater susceptibility to IR actions.[2,22]

Iron-sulfur (Fe-S) clusters are ancient modular co-factors of proteins that are involved in many cellular processes, such as enzymatic catalysis, electron transport, and gene expression regulation. Mammalian cells contain two aconitases that contain Fe-S clusters; the mitochondrial enzyme (ACO_2) involved in the tricarboxylic acid cycle.[24] IR can affect Fe-S cluster, it is known that IR, such as X ray on catalytic redox metal ions (principally iron and copper ions), lead to the production of •OH radicals via Fenton and Haber-Weiss chemistry which can enhance damage and alters by decreasing ACO_2 enzyme activity. The effect of X Ray on aconitase activity has been described as a bystander cell's effect persistent that could be detected after 20 population doublings.[25] These results strongly suggest that perturbations on molecules associated to oxidative metabolism persist long after the radiation exposure and explain stochastics and later effect of IR.

The IRs, in addition to the ROS, generate nitrogen reactive species (NRS) analogous to these in terms of their actions, but here the mediator is the N_2 dissolved in the body fluids, and NRS generation also has a cell endogenous production of Nitrogen oxide, the enzyme nitric oxide (NO) sintetase. This allows generation of high amounts of nitric oxide radical (·NO). Although in general this is inert for all cellular constituents (except for the hemo group), it can react with superoxide anion (O_2^-), generating a highly reactive peroxy nitrite anion ($ONOO^-$), associated with a low selection of restricted reactivity to neighboring molecules, including its inability to act as a cellular messenger. Contrary to what happens with other ROS, the NRS have a much larger diffusion coefficient which allows them to reach longer distances from their place of origin.[26,11]

Paradoxically, all these ROS/NRS are also generated as a natural result of breathing atmospheric air (20% of O_2 and 79% of N_2), which would be the main cause for human aging. Both reactive species have the characteristic of generating an oxidative stress that can produce deep chemical reactions (addition to macromolecules) and generating the risks for long-term damages, especially when they interact with DNA molecules. These oxidative damages and the free radical additions to the DNA are the main cause for their nitrogen base mutations, which can also accelerate the aging process and probably interrupt the expression of tumor suppressor genes such as p53 and retinoblastoma, allowing the conversion to malignant cells when they suffer some of these types of genomic lesions.

Most cells that are not a direct target of the IR can be affected by them. This is the result of the indirect long-term action of IR which can sometimes be both intracellular and intercellular ways of its products,the reactive species (ROS/NRS), to generate a known the bystander effect. This is another form of oxidative damage, which includes the carbonylation of peptides and proteins, peroxidation of lipids, appearance of spontaneous genetic mutations by these "electronic shifts" leading to several biochemistry changes which can potentially cause neoplasia transformation [Figure 3].[11,13]

Other sources of oxygen radicals *in vivo*

Superoxide ions are also produced naturally in cells by metabolic catalysis and cell respiration. The capture of O_2 is coupled into cellular respiration in order to produce energy bioavailability substances from organic substrates that come from ingested foods which are transformed into high-energy phosphate bonds and production of energy forms available to generate and maintain cell metabolism. This mostly occurs within the mitochondria by the electron transfer mechanism, reducing O_2 to H_2O, with the intermediary production of superoxide ions ($\cdot O_2^-$) that by enzymatic dismutation also result in hydroperoxide (H_2O_2) molecules. The peroxisomal oxidation of fatty acids is also a source of H_2O_2 as well as the products of the enzymes of cytochromes, such as P450. Another source of free radicals is the phagocytic activity of immune cells, in which they use a mixture of radicals and oxidants such as the O_2^- ion, hydroxyls (·OH), hypochlorite ($ClOH^-$), and peroxy nitrites (NO_2^-)[27] Briefly, cellular systems develop natural systems to reach a sustainable and suitable cellular homeostasis.[23,28]

Evaluation of damage at different levels with laboratory techniques

Microscopic analysis: Histological analysis

Even though there have been great diagnostic advances, including recent advances in molecular pathology and molecular testing, the role of techniques such as the histological analysis of tissue sections, and subsequent assembly in paraffin and stains, cannot be overstated in addition to the role played by traditional morphologists and clinical consultants for gastroenterologists, colorectal surgeons, oncologists, and geneticists, which remains very important. Histology techniques and their interpretations remain a relatively simple, very strong, and determining tool to observe potential tissue damage. On the other hand, histological and morphological characterization is fundamental for cytogenetic characterization such as microsatellite instability, gene expression, and sequencing characterization of all analyses. These need the appropriate selection of tissue sections for these tests and mutation analysis for markers such as APC, KRAS, and BRAF, which would serve to interpret the results of these therapeutic and prognostic tests further and reach a clinical relevance that must be highlighted.[29,30]

Cellular and subcellular analyses: 1-comet assay

A classical technique to evaluate IR damage has been the comet assay (CA), which consists of performing an agarose gel electrophoresis, stained with acridine orange, propidium iodine, or other DNA dye, from an anticoagulated whole

blood sample or lymphocytes extracted and separated from the rest of the blood elements by the Ficoll density gradient technique. With it, small differences in DNA chain breaks and damages can be distinguished between different cell samples.[2,31] Dose–response and time–response curves were combined to assess the potential of the CA in radiation biodosimetry and were used to detect DNA double-strand breaks (DSBs) in lymphocytes caused by X-ray and other photon irradiations.[18] The CA is a rapid and sensitive microdosimetric technique, particularly useful in accidental radiation.[32]

In a traditional CA, DNA migration is measured in a series of cells in the sample (where they are observed as comets when migrating from one pole to another in an electrophoresis gel, with the aureoles of broken DNA material that follow to the main body of each cell). Samples for CA can also come from cells in cultures and blood samples from patients or from animals under experimentation.[33,34] The CA reflects the physical status of genomic DNA, whereas 53 BP1/γ-H2AX staining represents processes related to the biological response to DNA damage (e.g., phosphorylation/dephosphorylation and recruitment/release of DSB repair-related molecules such as 53 BP1 and γ-H2AX).[35,36]

Cellular and subcellular analyses: 2-micronucleous assay

Micronuclei (MN) have turned out to be well-characterized biomarkers for chromosomal damage. Because MNs are formed from fragments of acentric chromosomes or lagging chromosomes that do not migrate to the poles during anaphase, the determination of MN frequencies is a reliable method for the evaluation of an effector (physical–chemical) to induce structural and/or numerical chromosomal alterations. Measurements of MN frequency within a corpuscular or cellular population are generally accepted as a very laborious alternative based on the counting on slide aberration in classical structural chromosomes (CA).

The most common *in vivo* assay used to detect genotoxic effects is the *in vivo* study of MN in erythrocytes in mice or MN in leukocytes. This experimental assay has been used routinely for decades and has been typically characterized to evaluate the frequency of erythrocytes with NA residues in bone marrow or peripheral blood smear cells. In mice, as in humans, there are two subpopulations of red blood cells (RBCs), namely mature RBCs and immature RBCs (reticulocytes [RETs]), which can be measured by MN techniques. Depending on the treatment regimen, the RETs and mature RBCs are easily differentiated, respectively, by the presence and absence of residual NA, or MN in leukocytes, which can be made visible by suitable staining methods with acridine orange and evaluated with fluorescence microscopy. Due to the processes of production and maturation of RBCs, it is important to use adequate sampling time, for example by the quantitation

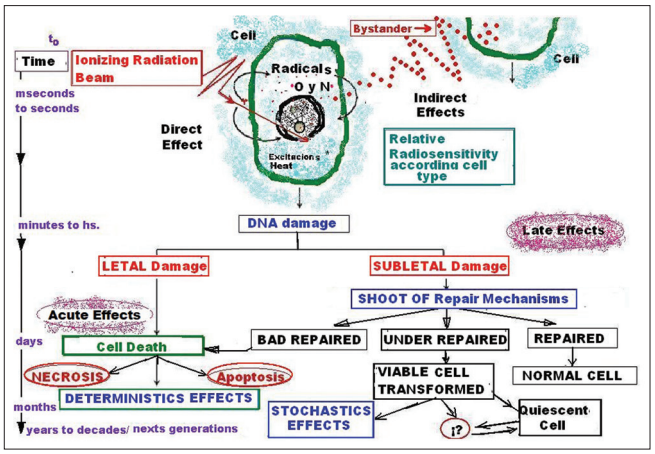

Figure 3: Ionizing radiation effects on live cells

and observation of RETs it can be evidenced the effects of recently induced damage within the 24 to 48 hours in humans or mice. RETs are possible to measure both in slides from bone marrow and peripheral blood representing each different time points from the exposure to the damage. In humans or mice, matured RBCs show accumulated damage from chronic exposure within 3- to 30-day window period; RBCs are typically measured by the observation of MN in blood smears in mice. Under continuous exposure, the frequency of micronucleated RBCs reaches a steady state in mice after approximately 30 days, which is half a period of the biological life of mouse RBCs. In rats, due to the rapid and efficient removal action by the spleen of the blood RBCs containing MN, the RETs have been measured in the bone marrow in order to completely eliminate the influence of splenic filtration on the frequency of MN-RET.[37,10] Many studies have evaluated the effect of IR using MN examination, the results showed by Fence, Killing, and Witt with chemotaxis.[37,10,38]

Molecular analysis: Detection of tumor gene expression

Different types of cancer are described by the accumulation of genomic alterations and are often caused by damages initiated by different factors, including physical types such as IR.[22,19] Therefore, genome sequence and structure analysis in cancer provides information to better understand the biology of cancer, its origin, its diagnosis, its prognosis, and the therapeutic strategies to apply. The application of second-generation DNA sequencing technologies (also known as next-generation DNA sequencing) allows for substantial advances in cancer genomics by analyzing the entire genome and the entire exoma (or analyze the known exons) and adopting a more complete approach such as transcriptome (i.e., sequencing the fully expressed genes) and protein activity to the complete genomes of cancer samples.[15]

These methods are facilitating an increase in the effi ciency and resolution of the detection of each of the main types of genome alterations and the identifi cation of central

altered pathways and facilitate the identification of possible therapeutic targets. Genetic studies include the detection of nucleotide substitutions, small insertions, as well as deletions, alterations of the number of copies, chromosomal rearrangements; and other alterations generated by physical, biological, microbiological, and chemical factors.[31,14,39,15]

When DNA damage creates double-stranded breaks (DSBs), it is always followed by the histone phosphorylation (H2AX). H2AX is a variant of the H2A protein family, which is a component of the octomeric histone in nucleosomes. It is phosphorylated by kinases such as ataxia telangiectasia mutated (ATM) and ATM-Rad3-related in the PI3 K pathway. This phosphorylated protein, γ-H2AX, is the first step in recruiting and localizing DNA repair proteins. These foci represent the DSBs in a 1:1 manner and can be used as a biomarker for damage. An antibody can be raised against γ-H2AX which can therefore be visualized by immunofluorescence through secondary antibodies.[35,40]

The creation of efficient new approaches for data analysis, in genomics, gene expression, transcriptomic, and proteomics, has the potential to dramatically accelerate biological and biomedical research, allowing a thorough analysis of genomes, transcriptomes, interactomes, and reactomes, to become affordable, routine, and widespread, instead of requiring significant and corporate efforts on a production scale.[41,42,15,7]

Using next-generation sequencing, researchers characterize a phenomenon called chromothripsis, whereby tens to hundreds of genomic rearrangements occur in a one-off cellular crisis. Rearrangements involving one or a few chromosomes cross back and forth across involved regions, generating frequent oscillations between chromosome copy numbers. These genomic hallmarks are highly improbable if rearrangements accumulate over time and instead imply that nearly all occur during a single cellular catastrophe. They found that one, or indeed more than one, cancer-causing lesion can emerge out of the genomic crisis. This phenomenon has important implications for the origins of genomic remodeling and temporal emergence of cancer.[7]

Cancer genome sequencing has identified chromothripsis, a complex class of structural genomic rearrangements involving the apparent shattering of an individual chromosome into tens to hundreds of fragments. An initial error during mitosis, producing either chromosome missegregation into a micronucleus or chromatin bridge interconnecting two daughter cells, can trigger the catastrophic pulverization of the spatially isolated chromosome. The chromosomal fragments are ligated into random order by DNA double-strand break repair during the subsequent interphase. Chromothripsis scars the cancer genome with localized DNA rearrangements that frequently generate extensive copy number alterations, oncogenic gene fusion products, and/or tumor suppressor gene inactivation.[43,44]

Strategies for the use of protecting mechanisms of radical actions with exogenous substances

Radioprotective substances are those that reduce the effects of IR in healthy tissues while maintaining sensitivity to radiation damage in tumor cells. Due to increased awareness about radioactive substances and their fatal effects on human health, radioprotective agents are now the topic of vivid research. Scavenging of free radicals is the most common mechanism in oncogenesis that plays an important role in protecting tissues from lethal effects caused by radiation exposure; therefore, radioprotective agents are also good anticancer agents. There are numerous studies indicating plant-based therapeutics against cancer and radioprotection. Such plants could be further explored for developing them as promising natural radioprotective agents with anticancer properties.[35,45] Cells possess a wide repertoire of enzymatic load exerting an important antioxidant role, where before the appearance of the oxidizing agents mentioned previously (due to the necessary and paradoxical existence of mechanisms of cellular respiration, which would also be mainly responsible for aging), they fulfill the important function of preventing the uncontrolled formation of reactive radicals such as ROS/reactive nitrogen species, from oxygen and nitrogen, respectively, but which are not 100% efficient and cause progressive cell aging and increase cancer risk.[33,23,28] Radioprotective agents may be classified as chemical (synthetic) or natural (plant derived).[32,35]

A radioprotector comprises a group of measures designed to ensure that people and their environment have protection against the harmful effects of IR. They are effective to rescue our bodies from wanted or unwanted radiations such as b, g, ultraviolet, or radionucleotide existents by NF, such as radioactive series of thorium and uranium.[32,35] Nevertheless, there are other radioprotective mechanisms which include the inactivation of protein kinase (PK)-C, NO, mitogen-activated PK, and downregulation of several other effectors responsible for molecular damage.

We can enumerate a few existing enzymatic natural scavenger systems. SOD catalyzes the reaction of 2 ($\cdot O_2^-$) + H_2O into O_2+ (H_2O_2), catalase is found in RBCs in high concentrations and catalyzes the degradation of 2 (H_2O_2) into 2 (H_2O) + O_2, and glutathione peroxidase also catalyzes the conversion of H_2O_2 into H_2O. Hydrophilic scavengers such as ascorbate acid, urate, ergo thionine, and glutathione are found in the nuclear, mitochondrial, and cytosolic compartments of cells and are responsible for the dangerous hydroxyl radicals (·OH). Some of the lipophilic scavengers known are the tocopherols (Vitamin E), flavonoids, carotenoids, and ubiquinol. Scavengers such as glutathione reductase and dehydroascorbate reductase first catalyze the reduction of the –SH groups into glutathione, a critical enzyme in the resistance to oxidative stress, to maintain a predominantly reducing medium in cells,

while dehydroascorbate reductase also has as function the maintenance of reduced mode of the –SH groups in the protein thiols. It is worth clarifying that all these enzymes and compounds act together, synergistically maintaining an interactive cooperation as an antioxidant network of an adaptive nature. They play a very important role in the regulation of expression activity at the transcriptional and posttranscriptional levels of high biological significance in terms of accommodation to the environment and the general regulation of the cellular homeostasis.[27,32,35]

More about radioprotectives, radioatenuants and radiomitigators

So far, in addition to the natural endogenous biological mechanisms mentioned above, several drugs have been reported to have radioprotection. These have been developed to reduce radiotoxicity, scavenge free radicals, and produce protection to nontumorous cells during radiotherapy in some types of cancers.[22,19,35]

The majority of these radioprotective compounds [Tables 1 and 2][28,46,47] fall into the category of antioxidants and have generated promising results by reducing xerostomia, mucus, pulmonary fibrosis, cystitis, inflammation, and alopecia, to mention a few observable effects with radiotherapy. Because they already have mitigating effects that are scientifically proven, they can be taken as reference to compare the anticipated results with respect to other bioactive substances or radical scavengers.[48,19]

The radioprotective effect of lactoferrin (LF) was studied in mice subjected to sublethal X-ray irradiation. The mice were randomly divided into groups called control (nonirradiated mice fed a standard diet without LF), IR (irradiated mice fed with a standard diet), and IR + LF (irradiated mice fed with LF). LF is an 80 kDa ironbinding glycoprotein, which is a component of exocrine secretions, including milk and saliva.[32]

Eckol, a component of brown seaweed *Ecklonia cava*, was tested against the high-energy photons which induced damage *in vivo*. Eckol demonstrated a significantly decreased mortality of lethally irradiated mice.[49]

Essiac tea is a combination of eight natural herbs that have proven to be a massive antioxidant and DNA protector. In a study published in 2005, Essiac was put to test with different types of free radicals. Fenton reaction (Fe^{2+} + $H_2O_2 \rightarrow Fe^{3+}$ •OH + OH^-) and chromium oxidant Cr(VI) in RAW 264.7 cell lines were used to produce hydroxyl radicals (OH^-), xanthine/xanthine oxidase system was used to produce superoxide radicals (O_2^-), and the same hydroxyl radicals (OH^-) from previous reactions were used to cause cell membrane damage to initiate lipid peroxidation in order to measure malondialdehyde (MDA) production. In summary, the study indicated that Essiac is an OH^- and O_2^- scavenger, which acts as a protector from cell membrane damage by inhibiting lipid peroxidation caused by the ROS and free radicals and protects DNA from OH^- radicals produced from Fenton reaction. All these mitigants and protectors share properties common to anticancer agents. We must take into consideration that lipid peroxidation can cause a cascade effect of lipid-derived radicals, which produces additional damage to cell membranes. MDA and other aldehyde groups, which are by-products from lipid peroxidation, may also produce DNA damage, but are inhibited by Essiac.[45]

Table 1: List of some known radioprotective substances

Amifostine
Nitróxides (tempol)
Antioxidants (glutathione, lipoic acid, Vitamin A, Vitamin C y Vitamin E, SODs)
Cisteín and Cisteamin
Melatonin
Novice radioprotectors (tetraciclines y fluorquinolones)
Curcumin

SODs: Superoxide dismutases

Table 2: List of some known radiomitigant substances

Palifermin
Halofuginone
TGF-β
KGF
ACE inhibitors
COX-2 inhibitors/NSAIDS
Essiac tea - 8-herbal mix

TGF-β: Tumor growth factor-beta, KGF: Keratinocyte growth factor, ACEs: Angiotensin-converting enzymes, COX-2: Cyclooxygenase-2

Conclusions

Exposure to IR is common to certain people such as professionals handling radioactive materials, patients undergoing radiodiagnostics and radiotherapy, or millions of people who travel by air who are scanned with X-rays every day. According to a report, 22 million people in the world are cancer patients and 6 million die of the disease. Although IR is indirect, it may trigger mutation in healthy cells, which further induces molecular alterations. It is known that IR generates free radicals from cytoplasmic water and this ultimately induces lesions and DNA damages. These damages may lead to neoplasm in normal and healthy cells because of the close relationship between radiation and cancer.[32,35]

IRs are carcinogenic, the ones that not only we can find in the environment, but also in some professional practices, among others. The DNA information of an individual is distributed in 23 pairs of chromosomes: 23 inherited from the father and 23 from the mother. In addition, one must take into account the mtDNA or DNA found within these subcellular organelles.[31,26] The fragments that have the information are the genes, which control the

growth, development, and cellular replication until their death. Nevertheless, any failures, mutations, or errors that occur within them can lead to genomic instability and promote the development of neoplasm in any individual, particularly, where the immune system also participates. This genomic damage can now be evaluated with a range of reliable and relatively inexpensive laboratory assays and tests to be carried out in populations where potential damage is suspected. The likelihood of occurrence of these types of cells increases with exposure to carcinogenic factors, with IR being one of many. The mechanism by which they induce these genetic instabilities is through the generation of highly reactive molecules such as ROS and NRS, which also participate in the aging process of our body. Therefore, with these free radicals produced during cellular respiration, and taking into account that although there is natural compensation to their presence, there should be a therapeutic strategy with the administration of antioxidants (radiomitigants or radioprotectors) for those populations with the greatest risk of being in contact with IR sources, as well as those people suspected of having a greater risk than the general population of generating some form of cancer and accelerated aging process.

IR causes consequential injuries to biological systems. Therefore, it is a necessity to formulate such dynamic pharmaceutical radioprotectors that can render protection to people against destructive and damaging outcomes of IR.[32,35]

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Conflicts of interest

There are no conflicts of interest.

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