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Cellular Oxidative/Antioxidant Balance in γ-Irradiated Brain: An Update

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Abstract: Both epidemiological and experimental data indicate that ionizing radiation (IR) may disrupt developmental processes leading to deleterious effects on brain functions. A central role of reactive oxygen (ROS) and nitrogen species (RNS), as important mediators in neurotoxicity and neuroprotection, has been demonstrated. Primary ionization events triggered by IR are amplified and propagated by mechanisms involving ROS and RNS, which activate several signaling pathways leading to final radiation effects. The immature and adult brain display clear differences in the way they respond to insults. Moreover, a great deal of attention is being focus on the limited antioxidant capacity and the particular lipid composition of cell membranes of the developing brain that render it more vulnerable to oxidative stress. The goal of this review is to summarize the current knowledge on the role of alterations in the balance between oxidative/nitrosative stress and antioxidant capacity in the pathways involved in cellular radiation response, with particular focus on the possible therapies proposed to limit radiation-induced effects in the brain.

Keywords: Antioxidant protection, fetal brain, gamma irradiation, nitrosative stress, oxidative stress.

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1. INTRODUCTION

The initial events when photons strike the matter and interact with the atomic nuclei or electrons (e^-) are ionization and excitation of atoms and molecules of the medium along the path of ionizing particles. Such physical disturbances involve a complex series of physicochemical, chemical and biological reactions that could act at several levels (such as molecular, subcellular, and cellular). The probability of damage depends on complex signaling events within and between cells [1]. Molecular oxygen (O_2), that represents 21% of the composition of the atmosphere, is essential to life, but it is also toxic because reactive oxygen species (ROS) are constantly formed as respiration by-products (by partial reduction of O_2) and by other pathways. The term ROS include free radicals, such as atomic oxygen ($O^{\bullet}$), superoxide anion ($O_2^{\bullet-}$), and hydroxyl radical ($^{\bullet}OH$), and non-radical reactive species such as hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$) [2, 3]. Nevertheless, as nature has been exposed to ROS for two billion years, selected mechanisms have been developed to allow biological systems, not only to live but also to use them, in multiple functions. Thus, ROS toxicity depends on their concentration and the cellular scenario in which they are produced [4]. On the other hand, N_2 represents 79% of composition of atmospheric air. Nitric oxide ($^{\bullet}NO$) is formed when N_2 combines with O_2. A double bound (σ-π) is formed and an unpaired e^- delocalized over the molecule and defines its nature as free radical [3]. When the $^{\bullet}NO$ reacts with the $O_2^{\bullet-}$ ($k = 6.7\ 10^9\ M^{-1}\ s^{-1}$) produces peroxynitrite ($ONOO^-$) [5, 6]. Reactive nitrogen species (RNS), as well, could lead to cellular damage by nitration, nitrosylation and finally to lipid peroxidation [3].

Enhanced cellular production of ROS has been observed after exposure to both, high and low lineal energy transference (LET) radiation [7], and it has been described as a very early and persisting event [8]. Radiation-induced changes in the cellular oxidative status may play a role in proliferation, differentiation and in the initiation of irreversible cell injury [9, 10]. In fact, enhanced cellular generation of ROS and RNS has been observed after exposure to low radiation doses and could lead to cellular amplification of signal transduction and further molecular and cellular radiation-responses [11]. Moreover, in the last few years a growing evidence of a crucial role for $^{\bullet}NO$ as a neuroprotector has been accumulated in the literature [12]. The goal of this review is to summarize the role of ROS and RNS in the amplification of radiation-induced pathways, and of the antioxidants with particular focus on their effects involving cell damage and protection in brain.

2. GENERAL FEATURES OF ROS AND RNS BIOCHEMISTRY

2.1. Reactive Species

ROS formation is a well established event in aerobic cells, as was mentioned previously. Between 1 to 2% of the consumed O_2 leads to the generation of these species from the leaking in the mitochondrial electron transport chain [3,

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13]. When O_2 accepts one e^- the primary product is $O_2^{\bullet-}$. This reaction can occur at the complex III in the respiratory chain or by the activity of the cytochrome P_{450}-depedent oxygenases, and oxidases such as NADPH and xantine oxidases. The reception of the second e^- leads to the formation of H_2O_2, and with the incorporation of another e^- the molecule decomposes to generate $^{\bullet}OH$. The subcellular structures such as, the mitochondria, the endoplasmatic reticulum, the peroxisomes, the plasma membrane, and several enzyme activities are responsible for ROS generation by partial reduction of O_2 [3].

In mammalian systems, $^{\bullet}NO$ can be produced by the activity of the $^{\bullet}NO$ Synthases (NOSs), a family of cytosolic enzymes. NOSs catalyze the conversion of L-Arginine to L-citrulline and $^{\bullet}NO$ consuming O_2 and NADPH [14]. In cell types that contain constitutive NOS isoforms (cNOS), NO is produced in relatively low amounts for short periods and elicits mostly direct effects. High concentrations of NO are produced via inducible isoforms of NOS (iNOS) thus the indirect, and often pathological, effects of NO are exhibited [15]. An enhanced generation of $^{\bullet}NO$ may lead to the increase on the steady state concentration of RNS. $^{\bullet}NO$ has an important role in the central nervous system (CNS), as it can function as a neuromodulator [16]. As a diffusible gas, it is involved in various mechanisms of synaptic plasticity in the brain and cerebellum, including those that play a key role in learning and memory [17, 18], and it is also involved in the regulation of the vascular tone and basal brain microcirculation [19]. Although constitutively expressed isoforms of NOS comprise neuronal (nNOS) and the endothelial NOS (eNOS), several studies involving prenatal insults in the developing brain have reported the alteration of nNOS expression [20].

2.2. Iron (Fe) Role in Radical Generation

Fe is essential to the brain function since it is a cofactor in numerous tyrosine enzymes and tryptophan hydroxylases, which are involved in the synthesis of neurotransmitters. It is also essential for the synthesis of lipids and cholesterol, which are substrates for the myelin synthesis [21]. Thus, Fe is a critical component in neurotransmission, myelination and cell division [22]. Fe can be found in two oxidation states: ferrous (Fe^{2+}) and ferric (Fe^{3+}) and this property makes it able to participate in reactions that cover a wide array of the cellular biochemistry [23], including DNA synthesis, e^- transport and cellular respiration [24]. Nevertheless, Fe is a suitable catalyst for the production of intermediates of the partial reduction of the highly reactive O_2 [25]. $O_2^{\bullet-}$, not a particularly potent oxidant, acts as a reducing agent reacting with Fe^{3+} leading to the appearance of Fe^{2+} (reaction 1) and generating, indirectly, $^{\bullet}OH$. Thus, only catalytic amounts of Fe are necessary to generate $^{\bullet}OH$ [25] (Fenton reaction).

Fe toxicity may arise both, from Fe^{2+} reacting with H_2O_2 and with lipid hydroperoxides (ROOH). The reaction of Fe^{2+} with H_2O_2 can lead to the production of oxoferrous compounds (FeO^{2+}) (reaction 2), which are powerful oxidants that can promote damage to biomolecules and, ultimately, lead to cell death [26, 27].

$$Fe^{3+} + O_2^{\bullet-} \longrightarrow Fe^{2+} + O_2 \quad (1)$$

$$Fe^{2+} + H_2O_2 \longrightarrow FeO^{2+} + H_2O \quad (2)$$

Besides, Fe toxicity may also result from its reaction with the ROOH (reaction 3). The alkoxyl radicals ($RO^{\bullet}$) produced during this reaction are responsible for lipid peroxidation, as shown in reactions 4 to 6.

$$ROOH + Fe^{2+} \longrightarrow Fe^{3+} + RO^{\bullet} + OH^- \quad (3)$$

$$RO^{\bullet} + RH \longrightarrow ROH + R^{\bullet} \quad (4)$$

$$R^{\bullet} + O_2 \longrightarrow ROO^{\bullet} \quad (5)$$

$$ROO^{\bullet} + RH \longrightarrow ROOH + R^{\bullet} \quad (6)$$

Other reductants, such as ascorbate (AH^-) and glutathione, may reduce Fe^{3+} generating Fe^{2+} [28, 29]. Reaction 7 shows the ascorbyl radical ($A^{\bullet-}$) production:

$$AH^- + Fe^{3+} \longrightarrow Fe^{2+} + A^{\bullet-} + H^+ \quad (7)$$

The pool of available Fe ions, called labile iron pool (LIP), is the crossroad of metabolic pathways of compounds containing Fe. The LIP term was proposed in 1946 by Greenberg and Wintrobe (1946) [30] and later by Jacobs in 1976 [31] as "a transient Fe deposit ". Operationally, it is identified as a “chelatable” pool that includes both ionic forms of Fe (Fe^{2+} and Fe^{3+}) associated with different populations of ligands, such as organic anions (phosphates and carboxylates), polypeptides and surface components of membranes (functional groups, the polar part of phospholipids) [32]. In biological systems, the LIP refers to the presence of catalytically active Fe, and clinical aspects that control the damage dependent on Fe overload requires the use of Fe chelators. Among the pharmacological issues, administration routes, ability of the compound to reach the target site, and its toxicity should be considered. For instance, clinical researchers are currently studying the designing of Fe chelators with the efficiency of deferoxamine (DFO), but that could be administered orally (instead of by injection), to meet critical goals such as good performance and comfort for the patient [33]. Elevated levels of Fe^{2+} found in CNS cells can promote damage because they enhance ROS generation [34]. Also, in the adult brain it was demonstrated that oxidative stress is implicated in the pathogenesis of many neurodegenerative and neurological diseases, such as Alzheimer's, Parkinson's and Huntington's disorders [35], although the etiology of these diseases is still unknown.

2.3. Antioxidant Defense System

The enzymatic and non-enzymatic antioxidants are aimed to keep the concentration of the reactive species in a controlled steady state values. Enzymatic protection is represented by the activity of catalase (CAT), thioredoxin reductases (TRs), glutathione peroxidases (GPxs), and superoxide dismutases (SODs) [36]. The function of CAT is to remove H_2O_2 within cells producing H_2O and O_2. CAT activity is present in most organs, especially in the liver, but only small amounts of this enzyme can be found in the brain, heart and skeletal muscle [37]. CAT activity in mammals is mostly located in peroxisomes and in a minor proportion in mitochondria [38]. TRs also convert H_2O_2 to H_2O and O_2,

such as CAT [39]. GPxs are cytosolic enzymes which catalyze the oxidation of a low molecular weight thiol, glutathione reduced form (GSH), to glutathione in its oxidized form (GSSG) reacting with H_2O_2 and generating H_2O [40]. In mammals, GPx exhibits high activity in the liver, moderate activity in the heart, lungs and brain, and low activity in muscle. SODs are a family of enzymes containing Cu, Zn or Mn. These enzymes catalyzed the disproportion of $O_2^{\bullet}$ generating H_2O_2 and O_2 [41]. Also in mammals, the CuZn-SOD activity was detected in nucleus [42], in the cytoplasm and outer mitochondrial space [43]. The reaction catalyzed by the enzyme Mn-SOD matches with the reaction catalyzed by CuZn-SOD, but its activity appears to depend strongly on the pH [41]. SOD antioxidant activity depends on its coordinate action with CAT activity.

The non-enzymatic antioxidants include AH^-, GSH, proteins that transport and storage transition metals (such as transferrin and ferritin), metallothioneins, carotenes, α-tocopherol (α-T), and flavonoids. The non-enzymatic antioxidants are the first defense against oxidative damage in the extracellular compartment [44]. The AH^- is synthesized from glucose in plants and most animals, except humans and other primates, which require the incorporation of AH^- within the diet (as vitamin C). The most important feature of the AH^- is its ability to act as a reducing agent. Donation of one e^- by AH^- produces $A^{\bullet}$, which can be re-oxidized to generate the dehydroascorbate (DHA). AH^- also participates in the reduction of Fe^{3+} to Fe^{2+} (reaction 7) facilitating Fe absorption by the intestine [45, 46]. Thus, AH^- can directly act as an antioxidant by reacting with aqueous peroxyl radicals or indirectly by restoring the antioxidant properties of other antioxidants, such as α-T. In addition, the deactivation of aqueous free radicals or oxidants by AH^- lowers the attack on other important cellular components, such as proteins and nuclear material [47]. The antioxidant synergism of vitamin E and vitamin C has been recognized and extensively studied. The key step is the reaction between the tocopheroxyl radical and vitamin C introducing a lipophilic chain in position 5 or 6 in vitamin C [48].

As previously indicated, GSH is the substrate of GPx and GSH is able to hijack $^{\bullet}OH$ and $^1O_2$. The safe storage and transport of ions of transition metals, such as Fe and Cu, is a way of preventing damage due to these species formation [21, 49]. On the other hand, both, α-T (highly soluble in lipids), and flavonoids (a group of polyphenols) can reduce themselves, oxidizing free radicals and producing very stable radical intermediates that terminate free radical chain reactions since they can not continue it.

3. EFFECTS OF γ-RADIATION ON THE BRAIN

Ionizing Radiation (IR) is used in medicine (diagnosis and treatment) and industry (technology) [50]. The primary source of ROS in cells exposed to radiation is the radiolysis of H_2O, during which $^{\bullet}OH$, H^+ and e^- are produced [51] (reactions 8 to 10)

$$H_2O + \gamma\text{-radiation} \longrightarrow H_2O^+ + e^- \quad (8)$$

$$H_2O^+ + H_2O \longrightarrow H_3O^+ + {}^{\bullet}OH \quad (9)$$

$$e^- + H_2O \longrightarrow e^-_{aq} \quad (10)$$

Since there is O_2 in the medium, the reactions 11 and 12 can occur:

$$O_2 + e^-_{aq} \longrightarrow O_2^{\bullet-} \quad (11)$$

$$H^+ + O_2^{\bullet-} \longrightarrow HO_2^{\bullet} \quad (12)$$

ROS, generated after irradiation, can produce downstream changes leading to damage that can manifest itself after months or even years from the initial irradiation event [52]. In addition to this indirect damage, there is a direct effect caused by γ-ray photons that are absorbed by macromolecules. For instance, single strand break (SSB), double strand break (DSB), cross-links, clustered base damage, and mismatch repair (MMR) can be produced in DNA. These alterations may affect the ability of the cell of transcribing the genes encoded by affected DNA [53]. Thus, the cell nucleus is the most sensitive cell structure since the genetic information is stored there, and the alterations described can lead to apoptotic cell death within 48-72 h [52]. Even though there are mechanisms for repairing and adaptation to irradiation that enable the cellular growth in a medium exposed to a natural background radiation, irradiated cells may either continue their normal functions or be transformed by losing the control mechanisms for multiplying [51].

The brain is one of the metabolically active organs of the body and the CNS utilizes approximately 20% of the total O_2 uptake [54]. Mainly, exposure of CNS to high levels of IR usually happens in the treatment of patients with primary or secondary brain tumors receiving cranial irradiation following surgical resection [52]. The brain function is affected by IR exposure producing long-term functional deficit such as cognitive, visual, and motor impairments [10]. Besides, IR produces long term learning and memory deficits, especially in children [9, 55]. Cognitive deficits might be due to deteriorated neurogenesis within the hippocampus, which is a key factor for learning and memory pathways [9, 56]. Several studies have shown that IR induces the loss of neural precursor cells (NPCs) in the hippocampus and subventricular zone in a dose-dependent way, probably due to the generation of $^{\bullet}OH$, $^1O_2$ and H_2O_2 in cells [57, 58]. The decline in brain functions not only is due to the production of ROS, but also to an acute inflammatory response after exposure to IR. Denham and Hauer-Jensen (2002) postulated that the overexpression of pro-inflammatory mediators could be crucial for radiation-induced normal tissue injury [59]. Besides, Moravan *et al*. (2011) have observed multiphasic inflammatory changes represented by increased transcript levels of inflammatory cytokines, along with glial and endothelial cell activation after cranial irradiation [60]. Elevation of cyclooxygenase (COX-1 and COX-2) activity, prostaglandin E2 (PGE2) synthesis, expression of adhesion molecules, tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), monocyte chemoattractant protein-1 (MPC-1) were also observed in irradiated brain [61-65]. Irradiation of mouse microglial (BV-2) cells, significantly increased activation of activator protein-1 (AP-1), nuclear factor-κB (NF-κB), and the cAMP response element-binding protein (CREB), within 24 h after γ irradiation [65, 66].

4. EFFECT OF γ-RADIATION ON THE DEVELOPING BRAIN

Thousands of pregnant workers and patients are exposed to IR annually. This exposure, not dangerous for many patients, may be critical for others. In fact, developing brain would be highly radiosensitive due to immaturity of their antioxidant defenses, the high content of Fe, and the high proportion of proliferating cells [67]. Thus, IR may be an important risk factor for the child yet unborn [68].

The high polyunsaturated fatty acids content that can be easily oxidized makes the CNS particularly sensible to ROS [54]. Moreover, the developing CNS is particularly sensitive to irradiation due to certain intrinsic features, such as: i) its structural complexity, ii) its long period of development, iii) the requirement of an appropriate location of cells for their correct function, and iv) the inability of neurons to proliferate once their final location in the developing cortex is reached [68].

Human gestation, completed between 37 and 42 weeks, shows three periods: (i) the pre-implantation phase (0 to 8 days of gestation, DG), (ii) the embryonic period (8 to 56 DG), and (iii) the fetal period (56 DG until the end of pregnancy) [68, 69]. The most sensitive period to radiation is the pre-implantation period where the outline of the germ layers that will give rise to different tissue systems is generated. Thus, irradiation at this stage of development usually results in the loss of the embryo. Yamada and Yukawa (1984) observed that the lethal dose 50 (LD50), when fecundation is preformed *in vitro*, is reduced from 1.5 Gy to about 0.3 Gy if exposure is performed 4-6 hours after the entry of sperm to the oocyte [70]. On the other hand, the fetal stage when growth, displacement and histological differentiation of organs occur, is the most sensitive period to irradiation leading to malformations, particularly in the brain, skeleton, eyes, teeth, and genitals [71]. The severe mental retardation (SMR) is the most important observed effect [72]. Another vulnerable period occurs from the 16th and the 25th gestational week, which corresponds to the beginning of synaptogenesis [73, 74]. The exposure of the fetus to 1 Gy between the 8th and 15th week after conception reduced intelligence quotient (IQ) in 30 points in children that also face a 40% probability of being born with SMR [68]. A reduced IQ was reported after exposure to fetal doses below 100 mGy, and SMR was observed with doses of about 500 mGy or higher [68].

It has been shown that γ radiation may promote an increase in NOS activity followed by an increase in $^{\bullet}$NO production in mammalian cells from liver, lungs, kidneys, intestine, brain, heart and bone marrow [75] In the rat at 17 gestational day of age the great majority of cortical cells stain to nNOS [76] with a low NOS activity in the brain [77]. Using different insults, including irradiation, an increase of nNOS activity and NO production have been reported in the brain and other systems from a few minutes to 1 h after the injury [78]. Moreover, Gisone *et al*. (2003) found a significant increase in the $^{\bullet}$NO steady state concentration 1 h post-irradiation (pi) in homogenates of fetal brain. The authors proposed that this $^{\bullet}$NO increase could promote protection against oxidative stress in the developing brain since there were observed neither alterations in the oxidative stress index $A^{\bullet}/AH^{-}$ (Table **1**) nor in the lipid radical content, after exposure *in vivo* to 1 Gy γ irradiation [79].

In rats, Robello *et al*. (2009) have shown that γ radiation *in utero* increases the total Fe content in both, the fetal whole brain and the maternal plasma (Table **1**) [80]. It was postulated that *in vivo* γ irradiation leads to an increase in Fe content in the interstitial fluid of the surrounding neurons. This change was produced by an increased Fe traffic in the blood through the placenta from the mother to the fetus. In this scenery, this effect on total Fe in the brain could increase the LIP in the cytoplasm (Table **1**) before it would be distributed among the cellular components, such as mitochondria and other organelles requiring the metal to fulfill their functions. Under these conditions, since an increase of the NOS total activity and in the $^{\bullet}$NO steady state concentration were observed (Table **1**), the produced $^{\bullet}$NO

Table 1. Main parameters measured in developing CNS exposed to irradiation.

Measurement	Control brain (17 GD)	Irradiated Brain - 1 h pi (17 GD)	References
[Total Fe] (pmol mg^{-1} FW)	60 ± 5	140 ± 6 *	[80]
[LIP] (pmol mg^{-1} FW)	4,1 ± 1.4 (6.8%)	4.8 ± 0.9 (3.4%)	[80]
[LIP maternal plasma] (μM)	1.9 ± 0.3	3.7 ± 0.7 *	[80]
[$A^{\bullet}$] (pmol mg^{-1} FW)	34 ± 5	35 ± 2	[79]
[AH^{-}] (pmol mg^{-1} FW)	437 ± 48	420 ± 40	[79]
[$^{\bullet}$NO] (nmol mg^{-1} FW)	37 ± 4	77 ± 5 *	[79]
Total NOS (pmol/g/30min)	39 ± 5	59 ± 5*	[79]
[$A^{\bullet}$]/[AH^{-}] (x 10^{-2})	7.1 ± 0.6	8.4 ± 0.9	[79]

Note: All values are expressed as mean ± SDE.

*Significant (P <0.05) when compared with the control group.

could bind Fe from the LIP resulting in the formation of Fe-nitrosyl complexes and finally, controlling the steady state level of the LIP. Therefore, a complex interaction between Fe and •NO could be operative as an endogenous mechanism contributing to the network of effects triggered to limit radiation damage [80]. In this regard, future studies should be developed to explore the possibility of designing therapeutic strategies to enhance cellular resistance to radiation damage. However, the survival of cells exposed to IR could be risky since they could suffer changes that would result in the lost of the control mechanisms. Thus, the brain would not develop normally over time and SMR, microcephaly and other disorders related to exposure to IR would appear.

5. USE OF ANTIOXIDANTS AS PROTECTION AGAINST γ RADIATION-DEPENDENT DAMAGE TO THE BRAIN

Steady state concentration of a compound is defined as the condition in which utilization and disappearance rates are equal. Thereby, an imbalance, either by an increase in the production of oxidative species (such as those caused by radiation exposure) or by a decrease in the antioxidant capacity, defines "oxidative stress" [3]. The reaction of the reactive species with macromolecules leads to alterations that could result in oxidative and nitrosative damage that can promote cell death, giving place to patho-physiological states. There are naturally occurring polyphenolic metabolites distributed throughout the plant kingdom and found in substantial amounts in fruits, vegetables, grains, nuts, seeds, tea, and traditional medicinal herbs [81, 82]. However, due to the polyphenolic structure, flavonoids have been found to possess both anti- and pro-oxidant actions as they can cycle between alcohol semiquinone and quione forms [83]. Forman et al (2014) [84] suggested that the oxidative activation of Nrf2 (related NF-E2 - related factor 2) signaling pathway is the main mechanism of action of nutritional antioxidants. These authors also proposed that the maintenance of “nucleophilic tone" is due to a mechanism called "para hormesis", that would provide a way to regulate the concentrations of the non-radical oxidants electrophiles to physiological (and hence no harmful) concentrations. Otherwise, antioxidant enzymes levels would increase, producing the detrimental of removal and repair systems of biomolecules [84]. As antioxidants, flavonoids are scavengers of ROS rendering beneficial effects. Besides, several recent studies have revealed that anticancer activities of flavonoids may be mediated through their pro-oxidant action [85]. Whether a flavonoid could act as anti- or pro-oxidant depends on its concentration, the source of free radical and the specific cellular conditions, and moreover on the flavonoid chemical structure [82, 86, 87]. Considerable attention had been paid to the formulation of antioxidants that could protect brain cells from the deterioration induced in a variety of brain disorders [34]. Lately, numerous antioxidant products have been tested to prevent the side effects of IR in the brain (Table **2**). The administration of flavonoids might protect NPCs from irradiation-induced cell death by their antioxidant properties [88, 89].

The treatment with the flavonoid baicalein (Fig. **1**) previous to the irradiation improves the viability of irradiated NPCs exposed to 16 Gy of γ-radiation. This effect was observed when mice were administrated with baicalein (10 mg/kg/day intraperitoneal (i.p.)) for 7 days starting on postnatal day 42, or when baicalein was applied directly on cultures of NPCs (1-10 μM) [55]. Moreover, the lost after whole body irradiation (WBI) of the Brain-Derived Neurotrophic Factor (BDNF, known to enhance hippocampal neurogenesis [90, 91]) was attenuated by baicalein, and the decrease in the phosphorylated form of cAMP Response Element-Binding (pCREB) was blocked. In addition, baicalein treatment before WBI significantly enhanced learning and memory performance relative to those

Table 2. Main features of flavonoids used in radiation protection.

Flavonoid	Origin	Effects	References
Baicalein	Roots of Huangqin, *Scutellaria baicalensis Georgi*	Antiallergenic, antibacterial and antioxidant, improvement of conditions generated by WBI	[106, 107]
Epigallocatechin-3-gallate (EGCG)	Green tea	Proposed as radiosensitizer of cancer cells	[94]
Resveratrol (RSV)	Grape skin	Cardioprotection, chemoprevention and antitumor activities, inhibition of self-renewal ability of GSCs	[95]
Septilin	Ayurvedic poly-herbal formulation (*Commiphora wightii, Trinospora cordifolia, Rubia cardifolia, Emblica officinalis, Saussurea lappa* and *Glycyrrhiza glabra*) 5555bvczb	Antioxidant, protections against several radiation effects	[100]
Nigella sativa oil (NSO)	*Nigella sativa* seed	Antihistaminic, antihypertensive, analgesic, anti-inflammatory, hypoglycemic, antibacterial, antifungal, antitumour, hepatoprotective, renal protective, antioxidant, decreases on RNS after IR	[99]

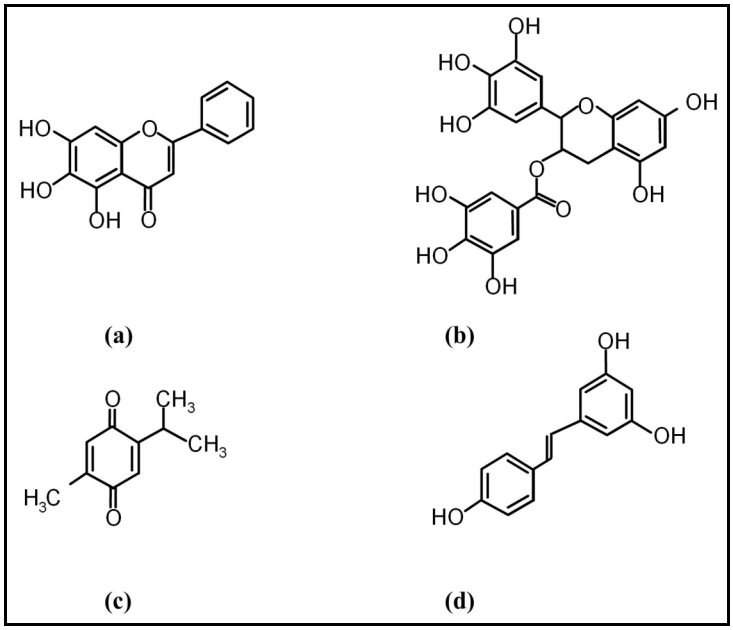

Fig. (1). Chemical structures of: (**a**) baicalein, (**b**) EGCG, (**c**) TQ, and (**d**) RSV.

observed in irradiated mice, and elevated pCREB immunoreactivity in the non-irradiated mice, which suggested that baicalein might have neurogenic properties [55]. Finally, since proliferation in the hippocampal dentate gyrus with 5 Gy of WBI was improved with the pretreatment with the flavonoid, these authors concluded that baicalein reduced the irradiation-induced ROS generation [55].

Epigallocatechin-3-gallate (EGCG) (Fig. **1**) is a non-oxidized catechin, and a water soluble member of the group of flavonoids [92]. Suganuma et al. (2011) [93] have proposed its potential use as chemo/radiosensitizer of cancer cells by showing the synergistic effects of the EGCG administration in different cancer treatments. Also, EGCG inhibited the expression of the Fe storage protein ferritin resulting in an increased availability of Fe^{2+} for catalyzing the Fenton reaction [94]. However, the flavonoid, that was described to induce the production of ROS and oxidative damage at high doses, could act as an effective antioxidant when used at low doses (high nanomolar to low micromolar levels) [94].

Glioma is a primary solid tumor in brain that is ineffectively treated by irradiation therapy because of its recurrence. The recurrence is due to the self-renewal capacity of glioma stem cells (GSCs). The plant phytoalexin, resverastol (RSV, Fig. **1**) is a non-flavonoid polyphenol tested by Sato *et al.* (2013) [95] on the tumorogenecity and the self-renewal capacity of GSCs. These authors found that RSV treatment inhibits this self-renewal ability of GSCs, inducing their differentiation [95].

Quinones, largely responsible for flower color, are ubiquitous in nature and highly reactive due to their aromatic rings with two ketone substitutions [96]. The *Nigella sativa* oil (NSO) from *Nigella sativa* seed contains thymoquinone (TQ) (Fig. **1**), which represents about 27-57% of the quinone constituents of this oil. TQ is an important active component present in the whole seeds or their extracts [97, 98]. Administration of a single dose of NSO (1 g/kg/day i.p.) or TQ (30 mg/kg/day i.p.) to rats γ-irradiated with 5 Gy produced a significant reduction to control values in NOS activity and in the levels of $^{\bullet}NO$ and $ONOO^-$ increased by this irradiation [99].

Septilin is an ayurvedic poly-herbal formulation containing *Commiphora wightii*, *Trinospora cordifolia*, *Rubia cardifolia*, *Emblica officinalis*, *Saussurea lappa* and *Glycyrrhiza glabra* [100]. Administration of septilin for 5 days (100 mg/kg) prior to radiation exposure resulted in (i) a significant increase in the activity of SOD and the total content of GSH, and (ii) a reduction of serum High-Density Lipoprotein-cholesterol (HDL), $^{\bullet}NO$ content, and malondialdehyde (MDA) levels, in hepatic and brain tissues [100].

Data in Table **2** briefly describe the main features of the mentioned antioxidant compounds. The diagram shown in Fig. **2** summarizes the direct and indirect effects of IR within the cell, as well as the effect of the listed antioxidants. The direct effects of γ irradiation include damage over DNA (SSB, DSB, MMR), proteins (oxidation of thiols, carbonyl formation, and activation/deactivation of various enzyme systems) and lipids (lipid peroxidation rendering membrane damage). The indirect effects of γ irradiation could be produced by the triggering of the increase in the steady-state concentration of ROS and RNS. Thus, the administration of these antioxidants is capable of limiting γ irradiation cellular deterioration acting at several metabolic points.

6. CONCLUSIONS

To fulfil the new drugs designing expectances, focus will be done in chemical-related aspects of the products used to

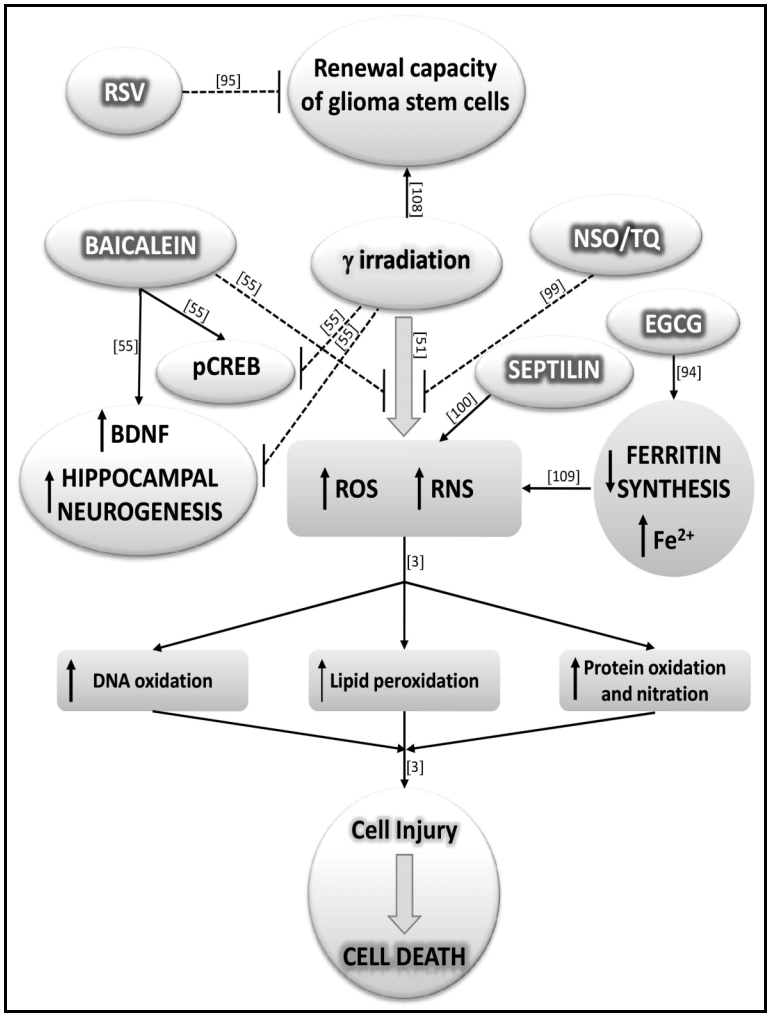

Fig. (2). Direct and indirect effects of IR within the cell and sites of action of enzymatic, non-enzymatic antioxidant, as well as sites of action of baicalein, EGCG, TQ, RSV and septilin. Numbers between brackets indicate the cited references describing this effect.

limit damage by radiation in the brain due to both radical and non-radical reactive species (such as peroxynitrite, hypochlorite, peroxides and lipid hydroperoxides, etc.).

An antioxidant is generally a molecule capable of slowing or preventing the oxidation of other molecules, by its ability to oxidize and reduce itself giving rise to a more stable free radical (by donating an electron or losing a proton) [101]. Generally, organic groups with double bonds allow the resonance of the molecule, stabilizing it [102]. Scavenging capacity of the reactive species by several compounds could help to avoid damage by radiation.

As shown in Figure **1**, the chemical structures of baicalein, EGCG, TQ, and RSV, render them as excellent antioxidants. When using a mixture of different chemical constituents, its scavenging activity could be due to a particular component, as well as to the interaction among several antioxidant molecules. Since the role of Fe and $O_2^{\bullet}$ in the initiation step of lipid peroxidation has been extensively discussed [25, 103], the ability of an antioxidant extract to limit $O_2^{\bullet}$ radical generation could be of interest in terms of its protective activity against radiation-dependent damage. Even though $O_2^{\bullet}$ radical is not particularly reactive, by removing it, the antioxidant extract would reduce or eliminate the formation of H_2O_2 and the reactive and toxic hydroperoxyl radicals derived from $O_2^{\bullet}$. Also by reducing the formation of H_2O_2, the generation of $^{\bullet}OH$ by the Fenton reaction would be decreased.

Moreover, an important factor in limiting the scavenging action of a compound is the accessibility of the antioxidant either to the cellular site of radical generation, or to the molecular targets of oxidative stress [104]. Thus, this factor should be considered during evaluation of the *in vivo* ability of an antioxidant. The efficiency of the incorporation of chemical compounds into the cell is a function of their lipophilicity [105], and the antioxidant activity of the extracts appears as depending not only on the structural features of their components but also on their location in the membranes. In this regard, it is important to point out that potential interactions between the components of the antioxidant extract with other nutrients, and the effects on absorption, transport, recycling and tissue specificity of other constituents could vary the effectiveness of the tested extracts in the whole animal.

These considerations must be taken into consideration for further biotechnological developments of protective antioxidants, which could have important applications in radiation therapy accompanied by free radical dependent cellular injury. Since any biologically active compound supplemented in the diet should appear in the target tissues in significant amounts to elicit bioprotective effects, future studies should consider interactions of dietary supplements with endogenous antioxidants, as well as tissue specificity, compartmentalization and concentration levels of the active compound/s in target organs after supplementation, to afford appropriate effectiveness *in vivo*. Unfortunately, the efficiency of the radiation treatment for cancer could be jeopardized by the antioxidant administration. The antioxidant treatment could both protect normal cells (critical factor to improve the quality of the radiation therapies) but also could preserve the integrity of cancer cells. Thus, a delicate equilibrium should be sought between both aspects.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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