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REVIEW

Free radicals, lipid peroxidation and antioxidants in apoptosis: implications in cancer, cardiovascular and neurological diseases

Carlos Kusano Bucalen FERRARI

Nutrition Department, Public Health School, University of São Paulo (USP), Av. Dr. Arnaldo 715, 2º andar, São Paulo (SP), Brazil, 01246-904; Tel.: ++ 5502111 30667701/5, fax: ++5502111 8526748, e-mail: carlokferrari@bol.com.br

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Reactive oxygen species (ROS) and free radicals promote redox reactions, altering biomolecules and damaging cell components. ROS can react directly with lipids, proteins, enzymes and DNA, altering them. ROS induce lipid peroxidation (LO), yielding peroxides, alcohols, aldehydes, ketones and cholesterol oxides (COs), most remarkably toxic, all of them present in animal cells and foods. It has been acknowledged that COs and oxidized LDL can provoke cell death of blood vessel macrophages, resulting in atherosclerotic plaque formation. COs are also cytotoxic to lymphocytes. During the evolution of living organisms, apoptosis, a "new" type of cell death, was conserved as a genetic programming of cellular suicide, triggered when cells were affected by small injuries to maintain the genome stability and save tissue integrity against damage. Oxidative stress can modify cell signaling pathways, inducing the expression of cell death genes and triggering enzymatic degradation of proteins, activating apoptosis. The antioxidant control of apoptosis in vital tissues (neuronal, cardiac, hepatic) should consider signaling mechanisms and gene expression triggered by ROS stimulation.

Key-words: apoptosis, calcium, cell signaling and gene expression, free radicals and lipid peroxidation, antioxidants, cellular pathophysiology.

Abbreviations: AA, arachidonic acid; Ac-YVAD-cmk, ac-Tyr-Val-Ala-Asp-CHO-chloromethylketone (protease inhibitor specific to the pro-ICE); AP-1, activator protein-1; APC, anaphase promoting complex; Bcl-2, oncogenic protein of human follicular lymphomas; Bax, protein of the Bcl-2 family; Caspases, cysteine-rich enzymes that clive peptides at aspartic residues (cysteine-containing aspases); CAT, catalase; CO, cholesterol oxide; DAG, diacylglicerol; DFF, DNA fragmentation factor; E2F-1, transcription factor-1; fMLP, N-formyl-L-methionyl-leucyl-L-phenylalanine; FR, free radical; GSH, glutathione; ICE, interleucin-1β-converting enzyme; IGF-1, insulin growth factor-1; INF-γ, interferon-γ; JNK/p38, a MAP kinase of SAPKs; LO, lipid oxidation; MAPK family, mitogen-activated protein kinase (a family of serine/threonine kinases, such as Raf, MAPK kinase kinase or MKKKs); MDA, malonaldehyde; NF-kB, nuclear factor kappa-beta; NAC, N-acetyl-cysteine (antioxidant); NO, nitric oxide; PAF, platelet-activating-factor; p53, cell-cycle check-point oncoprotein; PARP, poly(ADP-ribose) polymerase; PCD, programmed cell death; PDGF, platelet-derived growth factor; raf, a MAPK protein; ras/rac family, small G-proteins; rb-1, retinoblastoma oncoprotein-1; ROS, reactive oxygen species; SAPK, stress-activated protein kinase; SOD, superoxide dismutase; TGF-β, transforming growth factor-β; TNFα, tumor necrosis factor-α; z.VAD.FMK, z-Val-Ala-DL-Asp-fluoromethylketone (inhibitor of the protease caspase-3 or CPP32).

Introduction

The concept of apoptosis or PCD was introduced in 1972 by KERR and colleagues, although GLÜCKSMANN in 1951 and LOCKSHIN in 1974 have described the same phenomenon (WYLLIE, 1997). The apoptotic process takes part in a variety of physiological and pathological states since fetal development, T-cell depletion control, tumor regression, normal cell turnover, neuronal and cardiac tissue loss, infectious and parasitic diseases, until final differentiation of normal cells (BARCINSKI, 1998; BUTTKE & SANDSTROM, 1995; DELONG, 1998; MCCONKEY & ORRENIUS, 1994; OLIVETTI et al., 1997). Contrary to necrosis, in which there occurs leakage of cytoplasmic content, in apoptosis there is cytoplasmic condensation without membrane damage, which avoids inflammatory reactions. Besides, necrosis — a passive cell death — is caused by an exhausting injury and is characterized by swelling of the cell and its organelles and by a highly flocculated form of chromatin, which gives to DNA a smear appearance in agarose gel electrophoresis (GARDNER et al., 1997). Some cellular and molecular features that characterize apoptosis are summarized in Table 1.

Genetic programming of apoptosis

There are several factors involved in the activation or inhibition of apoptosis, but the genes play a special controller role. In the *Caenorhabditis elegans* worm there are at least three genes that have an inducing role in the apoptotic process: the Cell death-3 (Ced-3), the Cell death-4 (Ced-4) and the Cell death-10 (Ced-10) genes (FANIDI & EVAN, 1994; WYLLIE, 1997). The Ced-3 gene product is a homologous protein to the mammalian interleukin 1β-converting enzyme (ICE), a cysteine apoptotic protease. In this sense, the use of the Ac-YVAD-cmk tetrapeptide, a specific ICE/Ced-3 inhibitor, was able to block apoptosis in AK-5 tumor cells (ANJUM & KHAR, 1997). Unlike the Ced-3, the Ced-9 gene, which has some homology with the bcl-2 human proto-oncogene, is involved in the suppression of apoptosis through inhibition of ICE activation (FANIDI & EVAN, 1994; SAVILL, 1998; WYLLIE, 1997). On the other hand, the Ced-5 gene encodes a protein similar to the human DOCK180 that is responsible for the cytoskeleton rearrangement required for recognition and phagocytosis of the dying cells in the mentioned worm (WU & HORVITZ, 1998). Recently it was discovered that the human CD14 receptor plays an important role in the recognition and engulfment of apoptotic bodies (DEVITT et al., 1998). In mammalian cells there exist some genes responsible for production of the ICE-family enzymes or caspases, one of the last executioners of PCD events, that induce the cleavage of cellular proteins associated with cell cycle control, nuclear membrane

integrity (lamins), DNA repair and integrity (PARP, DNA-PK, DFF) and cytoskeleton and microtubules organization (APC, fodrin, actin, MARK) (DREWES et al., 1997; LIU et al., 1997; WYLLIE, 1997). The most important apoptotic genes in human cells are the cell cycle checkpoints-c-myc and p53 (FANIDI & EVAN, 1994)- although many more genes could be implicated. Therefore bcl-2, v-abl, other proto-oncogenes and certain viral genes can abrogate the apoptotic process, increasing cell survival (FANIDI & EVAN, 1994; WYLLIE, 1997). Bcl-2 can inhibit NF-kB leakage-induced apoptosis (HERRMANN et al., 1997A), as well as it is able to suppress the activity of caspases.

Nevertheless, there are other relevant factors in the management of cell death programs. A lot of cell physiological mediators, such as interleukins 2 and 4 (IL-2, IL-4), TGF-β, TNF-α and INF-γ are normally able to induce PCD (DELONG, 1998; FANIDI & EVAN, 1994; FESUS et al., 1996; ISLAM et al., 1997; WYLLIE, 1997). However, IL-1α, IGF-1, PDGF, thiols and other antioxidants usually inhibit apoptosis (DELONG, 1998; FANIDI & EVAN, 1994). Inhibition of the receptor for IGF-1 is an important strategy to control cancer cells through execution of apoptosis (BASERGA, 1995).

Cell signaling can be initiated through the interactions of hormones, cytokines, neurotransmitters and other molecules such as hydrogen peroxide (H_2O_2) with plasma membrane receptors. There is another possibility when the plasma membrane structure is damaged by energy sources (thermal, UV and ionizing radiation), chemical agents (xenobiotics and pollutants) and free radicals (BUTTKE & SANDSTROM, 1995; MONTEIRO & STERN, 1995; PALMER & PAULSON, 1997; SAMALI et al., 1996). All of them trigger a series of signaling systems involving complex kinase cascades such as protein tyrosine kinase, protein phosphokinases, protease cascades, lipid fragmentation products [from phospholipid degradation (by damaging agents or phospholipases)] and other biomolecules that act as secondary and intermediary messengers (cAMP, cGMP, Ca^{2+}), influencing transcription factors such as NF-kB, AP-1, ras, JNK/SAPK, E2F-1 and rb-1 that can modulate cell growth (mitogenic response) or apoptosis through their protein products (DELONG, 1998; FANIDI & EVAN, 1994; MONTEIRO & STERN, 1995; PALMER & PAULSON, 1997; SAMALI et al., 1996).

Oxidative stress and peroxidative reactions constitute important factors that can cause PCD in a number of pathophysiological processes, including HIV infection (BUTTKE & SANDSTROM, 1995). Thus we review some cellular and molecular mechanisms concerning the role of oxidants and antioxidants in the PCD process.

Oxidative stress, free radicals and apoptosis

An FR is any molecule that has one or more incomplete orbitals. Thus a FR can gain electrons, oxidizing another atom/molecule or lose them reducing an element. Some Reactive oxygen species (ROS) are FRs ($O_2^{\bullet -}$, $^{\bullet}OH$, $NO^{\bullet}$, $ONOO^-$) although others are not (H_2O_2, $^1O_2$). ROS and FRs promote redox reactions in biomolecules, damaging cell components. ROS can react directly with lipids, proteins, enzymes and DNA, altering them (FERRARI, 2000A).

Hydroxyl radical ($^{\bullet}OH$) is formed by the Fenton or Haber-Weiss reaction of hydrogen peroxide with superoxide anion in the presence of metals (mainly iron):

$$H_2O_2 + O_2^{\bullet -} \rightarrow OH^- + {}^{\bullet}OH + O_2$$

$^{\bullet}OH$ was capable to induce PCD in polymorphonuclear leucocytes since iron addiction to H_2O_2 provoked apoptosis, whereas deferroxamine and hydroxybenzil, two iron chelators, enhanced cell survival (ROLLET-LABELLE et al., 1998). In the same study, neutrophils were incubated with xanthine oxidase, which induces the production of H_2O_2 and $O_2^{\bullet -}$, or with glucose oxidase, which yields H_2O_2 only. After this, there was an increase in the PCD rate that was associated with reduced levels of GSH content and the PCD was inhibited by CAT, but not by SOD. Similar results were obtained with HIV-infected lymphocytes (DOBMEYER et al., 1997). Those authors also revealed a positive correlation between ROS production and PCD rate; and lymphocytes' depletion was associated with disease severity.

Another ROS that promotes apoptosis is represented by H_2O_2 (GARDNER et al., 1997; SAMALI et al., 1996). Hydrogen peroxide can provoke PCD in low concentrations (5 to 10mM) and necrosis in the highest levels (>10mM) (GARDNER et al., 1997). In the same study, the authors had observed that cycloheximide, emetine, aminobenzamide and calcium depletion were effective in the inhibition of H_2O_2-mediated apoptosis. In 25% of the cases of familial amyotrophic lateral sclerosis was detected the occurrence of single mutations in SOD1 gene (that encodes Cu/Zn-SOD) which enhance the activity of Cu/Zn-SOD to produce H_2O_2. Following this approach, WIEDAU-PAZOS et al. (1996) demonstrated that these CuZnSOD mutants promoted increasing production of H_2O_2 and apoptosis in a neural cell line. In this sense, JEWETT et al. (1999) had strongly demonstrated that reduction of Copper(II) by $O_2^{\bullet -}$ in the structure of Cu/Zn-SOD enzyme results in loss of Copper(I) and intensive activation of Fenton reactions, which culminate in damage to biomolecules.

Nitrogen dioxide can be reduced to nitric oxide ($NO^{\bullet}$), which reacts with $O_2^{\bullet -}$ yielding peroxynitrite ($ONOO^-$) (FERRARI, 2000A). MEβMER & BRÜNE (1996) found that four NO sources were capable to promote nuclear fragmentation, morphological changes and p53 accumulation in apoptotic macrophages, also that the best effect had been attributable to H_2O_2. Incubation of murine macrophages (RAW 264.7) and human intestinal epithelial (T84) cell lines with low concentrations

(100-300μM) of $ONOO^-$ induced PCD, whereas higher levels of $ONOO^-$ (>300μm) induced necrosis and previous or simultaneous treatment with L-ascorbic acid abrogated the PCD (SANDOVAL et al., 1997). In contrast to the apoptotic roles of peroxynitrite, nitric oxide has been capable to protect cells from TNF-induced apoptosis. One study revealed that the antiapoptotic role of NO is provided by its capacity to induce antioxidant systems, e.g. through the induction of Heat shock protein-70 expression (Hsp70) and formation of S-nitrosoglutathione and oxidized glutathione; both of them inhibited ROS and LO reactions in cultured hepatocytes (KIM et al., 1997). It's important to note that the majority of studies have demonstrated a pro-apoptotic role of NO (RICHTER, 1998). In view of those papers, we can hypothesize that low levels of NO cannot affect cell survival (once cell antioxidant defense systems are efficient), but higher NO levels are sufficient to induce programmed-killer pathways.

Lipid peroxidation products and apoptosis: from disruption of cell membrane until haemolysis and atherosclerotic plaque formation

One of the most important consequences of the oxidative stress is that it triggers the peroxidation of unsaturated fatty acids in phospholipids and sphingolipids of the cell's membranes, which results in a series of damages in the cell's structure and function (KUBOW, 1992; FERRARI, 1998; FERRARI, 2000A) as discussed below.

Lipid peroxidation modifies membranes and provokes the release of unstable hydroperoxides and final secondary products, such as aldehydes, ketones, esthers and polymers, most remarkably toxic (ESTERBAUER, 1993; FERRARI, 1998). ROS also produce cholesterol oxides from food or blood cholesterol. All these products are present in animal cells and foods (ESTERBAUER et al., 1991; FERRARI, 1998; FERRARI, 1999; KUBOW, 1992). In addition, AA metabolites, PAF, DAG and ceramides are also produced by LO reactions (CLUTTON, 1997; ESTERBAUER, 1993; FERRARI, 1998; ZIMMERMAN et al., 1995).

If hydroperoxides have been implicated in the pathogenesis of haemolytic anemia, bowel irritation, growth retardation, hepatic and renal degeneration (ESTERBAUER, 1993) and lymphoid tissue necrosis (OARADA et al., 1988) it is not strange that they also cause apoptosis (BUTTKE & SANDSTROM, 1995; CLUTTON, 1997; SAMALI et al., 1996). Because of their instability, hydroperoxides are broken to produce alcohols, esthers, ketones and aldehydes, especially MDA, 4-hydroxynonenal and alkenals. MDA is mutagenic, carcinogenic and cytotoxic, inducing necrotic cell death (CLUTTON, 1997; ESTERBAUER, 1993; ESTERBAUER et al., 1991; FERRARI, 1998). In this sense, JI et al. (1998) revealed 3 to 6-fold increase of MDA-deoxyguanosine DNA adducts in two

cell lines and cell cycle arrest induced by MDA, factors that contribute to the induction of the apoptotic process. It has been reported that MDA is capable of accumulating itself in the erythrocyte membrane, inverting its structure and exposing the internal side phospholipids to oxygen (JAIN, 1984), inducing haemolysis and coagulation (DRAPPER et al., 1984). This exposition of phosphatidylserine to the outer side of membrane can lead to PCD and it is useful for detecting apoptosis (YANG et al., 1998). 4-hydroxynonenal, another toxic aldehyde, was responsible for the programmed death of two neuronal cell lines (KRUMAN et al, 1997). It is important to note that oxidative fragmentation of phosphatidylcholine, also in low-density lipoproteins, generates PAF-like molecules that can induce signal transduction, inflammation and smooth muscle cell mitogenesis (ZIMMERMAN et al, 1995), which show the dual role of lipid disruption.

In recent years there has been an acknowledgment of the role played by injuries in the activation of sphingomyelinases and, consequently, of the sphingomyelin hydrolysis that generates ceramides, stress molecules capable of inducing apoptosis or cell proliferation (HANNUN, 1996; FRIEDMAN-HAIMOVITZ et al., 1997; MCCONKEY & ORRENIUS, 1994; SAMALI et al., 1996).

Although the roles of lipids in cell survival and death were not well established, HERRMMANN et al. (1997) have observed that bcl-2 and protein kinase C inhibited ceramide-induced apoptosis and that free AA also blocked the PCD in prostate carcinoma cell lines. They also showed that selective suppression of c-phospholipase A_2 or lipoxygenase activities could promote PCD.

Since the 70s it has been recognized that COs from oxidized foods or oxidized LDL (ox-LDL) particles can provoke cell death of blood vessel macrophages, resulting in atherosclerotic plaque formation (STEINBERG, 1988; HALLIWELL, 1995). COs are also cytotoxic and genotoxic to the cells (ESTERBAUER, 1993; KUBOW, 1992; SEVANIAN & PETERSON, 1986) and were able to suppress the cytotoxic activity of T cells against mastocytoma cells (KÜÇÜK et al., 1994).

In Natural Killer cells exposed to ox-LDL MALORNI et al. (1997) observed structural changes in the microtubular apparatus, significant decrease in TNF-α (-38%), IL-12 (-79%) and INF-γ (-95%) release, as well as a reduction in the GSH content, which resulted in a significant decrease of the cytotoxic activity of those cells. Experiments of AYALA-TORRES et al. (1997) demonstrated that 25-OH-cholesterol, a CO, promoted PCD in human leukemia cells and that mevalonate and z-VAD.fmk, a caspase suppressor agent, were able to partially restore cell survival. KINSCHERF et al. (1998) revealed that exposition to ox-LDL, ceramide and H_2O_2 of human macrophages resulted in a two to five-fold increase of PCD, which was associated with the expression of p53 and Mn-SOD mRNA, whereas the GSH content had decreased and bcl-2 expression had not been affected. On the other hand, previous treatment with NAC, an antioxidant, prevented all reported phenomena. ESCARGUEIL-BLANC et al. (1994) had observed that ox-LDL could induce two distinct pathways of Ca^{2+}-dependent cell death: apoptosis, when DNA endonucleolysis occurred and necrosis, in case of

proteolysis' existence. Considering the molecular role of ox-LDL, SATA & WALSH (1998) observed that endothelial cells were programmed-killed by ox-LDL through Fas, a type I surface protein of the TNF receptor family (TNFR) which binds to its ligand, FasL, initiating the apoptotic process. It was also reported that anti-FasL-antibodies were capable to inhibit PCD. Closing, ALCOUFFE et al. (1999) observed that ox-LDL induced apoptosis in three different lymphocyte populations. Since the role of LO, CO and FR in PCD are not still completely resolved, it is relevant to pay attention to the Ca^{2+}-dependent cytotoxicity suggested by SEVANIAN & PETERSON in 1986.

Oxidative stress, calcium influx, mitochondrial homeostasis and PCD

Ions play important roles in the control of cell functions. In this sense, Ca^{2+} physiological roles are very important. Beyond its neural and muscular functions, extracellular Ca^{2+} up-take or cytosolic Ca^{2+} permanence, both H_2O_2-induced, were associated with apical microvilli elongation (REID et al., 1997). It was observed that increase of Ca^{2+} influx through the N-methyl-D-aspartate (NMDA) receptor channels, due to the inhibition of Mg^{2+} extracellular up-take, resulted in glutamate release and neurological injury (ZHANG et al., 1996). However, K^+ current in neuronal cells may play a controller's role in Ca^{2+} signals for life or death. The increase in extracellular K^+ enhances Ca^{2+} influx through voltage-gated Ca^{2+} membrane-channels, which results in an intracellular cytoplasmic accumulation of Ca^{2+}, preventing cell death of non-damaged cells (YU et al., 1997). These authors demonstrated that loss of total intracellular K^+ with consequent enhancement of K^+ membrane efflux can mediate PCD and that massive Ca^{2+} cytosolic mobilization (Ca^{2+}-overload) to the outside, as well as to the inside of the cell membrane, led cells to apoptosis. Besides, Ca^{2+} participates in PCD via several pathways (CLUTTON, 1997; FESUS et al., 1996; SAMALI et al., 1996; WYLLIE, 1997): 1) Phospholipase C activation, which releases DAG and inositol-triphosphate and this induces Ca^{2+} release from nuclear membrane and endoplasmic reticulum; 2) Ca^{2+} can induce PKC; 3) The Ca^{2+}-dependent-poly-(ADP-ribose)-synthase is involved in the oxidative stress stimulation with massive expenditure of ATP; 4) Ca^{2+} activates endonucleases that fragment chromatin; 5) Ca^{2+} or Ca^{2+}-ionophores stimulates the expression of transglutaminases that induce protein cross-links, resulting in PCD; 6) Ca^{2+} chelators, calbindin, a Ca^{2+}-binding protein, or Ca^{2+} channel blockers can prevent PCD; 7) Ca^{2+} cytosolic stores can be controlled by bcl-2 protein.

It is important to note that OS provoke ionic disturbances that can risk cell survival. It was revealed that transfection of antisense oligomers directed against glutamylcysteine synthase, which resulted in abrogation of GSH production, was associated with increase of FR and intracellular Ca^{2+}, leading cells to PCD (JURMA et al., 1997). Membrane hyperpolarization, which depends on

intracellular Ca^{2+} and extracellular K^{+} concentrations, was induced by the xanthine-superoxide generating system, by the fMLP peptide and PAF. This event, which stimulates ROS release by macrophages, was inhibited by SOD but not by CAT (GAMALEY et al., 1998).

In spite of the above considerations, Ca^{2+} and ionic influx are not regulated only by membrane channels or endoplasmic reticulum, but also by mitochondria, the FR source (HALESTRAP et al., 1993). Indeed, endoplasmatic reticulum and mitochondria establish intimate contacts that permit Ca^{2+} influx from the 1st to the 2nd organelle (RIZZUTTO et al., 1998). Thus, depletion of intracellular Ca^{2+} pools in the endoplasmic reticulum by thapsigargin, t-butyl-hydroquinone and cyclopiazonic acid, three inhibitors of Ca^{2+}-ATPases, were able to induce apoptosis in two different lines of insulin-secreting cells (ZHOU et al., 1998). However, the same study showed that: 1) Ca^{2+} chelators and inhibitors of Ca^{2+} membrane channels did not prevent PCD; and 2) Inhibition of lipoxygenase metabolism partially restores cell survival.

A lot of studies took into consideration the role of the oxidative stress in mitochondrial leakage, cytochrome c and apoptosis. In *C. elegans*, a missense mutation in the cytochrome-1 gene, homologous to bovine succinate dehydrogenase (SDH-$cytb_{560}$), impairs the electron transference from SDH-cytochromeb560 to the Qsemiquinone transporter, resulting in massive accumulation of $O_2^{\bullet -}$ (ISHII et al., 1998). This feature can be associated with premature aging and certain neuropathologies like Leigh's syndrome, in which there occurs a SDH gene mutation (ISHII et al., 1998). But real proof of a PCD-cytochrome-c relationship was gave by ZHIVOTOVSKY et al. (1998). In a similar way, STRIDH et al. (1998) observed that tributyltin provoked loss of mitochondrial membrane potential with Ca^{2+} mobilization, concurrently with cytochrome-c release (from mitochondria) and caspase activation, which was similarly verified in respect of H_2O_2-induced PCD. The PCD induced by injected cytochrome-c or by Bax-induced cytochrome-c release were both prevented by caspase inhibitor and mitochondrial stabilizer- the bcl-2 (ROSSÉ et al., 1998; ZHIVOTOVSKY et al., 1998).

It has been demonstrated for some years that ROS and FR are both inducing and executor agents of apoptosis, once they are able to stimulate signal transduction pathways and mitochondrial permeability transition (JABS, 1999). This opening of mitochondrial megachannels results in mitochondrial disruption, Ca^{2+} release, cellular antioxidant consumption and amplification of ROS production (KRISTIÁN & SIESJÖ, 1998; JABS, 1999). These events are characteristic of the calcium-mediated ischemic cell death (KRISTIÁN & SIESJÖ, 1998). After ROS overload and mitochondrial damage, the free cytochrome-c will connect itself with the apoptotic protease activating factor-1 (Apaf-1) forming a complex that cleaves procaspase-9 to form caspase-9, which induces apoptosis through the executioner caspases 3,6 and 7 (FERRARI, 2000B), resulting in nuclear degradation and other PCD features (Table 1).

How and which antioxidants can protect the cell's life?

As described earlier, some physiological factors can induce or inhibit PCD. There is great evidence that a lot of agents are able to promote oxidative stress and antioxidant depletion resulting in PCD. It was demonstrated that TGF-β1 induced LO and inhibited glutathione peroxidase and CAT, promoting apoptosis (ISLAM, 1997). The suppression of GSH was associated with PCD in liver hemopoietic cell lines, but not in hepatocytes (YAMAMASU et al., 1997). On the other hand, ROS have been implicated in the p53-dependent apoptotic pathway (KINSCHERF et al., 1998), which can involve a controller of phosphatidylinositol-3-OH kinase, the p85 protein (YIN et al., 1998). DRUKARCH et al. (1998) observed that inhibition of glutathione synthesis resulted in an increasing rate of neuronal cell death. On the contrary, addiction of CAT and CAT plus SOD (more effective), but not SOD alone, restored the neuronal survival. Neutrophil's PCD was associated with enhancing the production of NO and with the decrease of phagocytosis, of glutathione peroxidase and CAT release (CURI et al., 1998).

In view of those studies, we understand that some other antioxidants can protect cell life. GSH, L-cysteine, ascorbic acid, α-tocopherol, NAC, trolox, butylated hydroxyanisol and ebselen can be used to prevent oxidative-induced PCD (BUTTKE & SANDSTROM, 1995; CHAU et al., 1998; CLUTTON, 1997; KINSCHERF et al., 1998; YAMAMASU et al., 1998). For example, ebselen, a selenium-containing antioxidant, diminished LO, limiting the rate of apoptosis in myocardial cells (MAULIK et al., 1998). Nevertheless, tea polyphenols, recognized antioxidants (RAMARATHNAM et al, 1995), were associated with H_2O_2-induced apoptosis and CAT abrogation in human cancer cell lines (YANG et al., 1998). This type of PCD role that is mediated by polyphenols is similar to those found in certain anticancer drugs like β-lapachone (a topoisomerase inhibitor) (CHAU et al., 1998) and quinones (QIU et al., 1998), as well as in natural-derived anticancer products (cruciferous vegetables, apples and garlic) (DELONG, 1998). The apoptotic properties of these products could be used in the anticancer therapy but loss of cells must be controlled with care.

Apoptosis: simply a result of oxidant/antioxidant imbalance?

In addition, it is necessary to emphasize that depending on type and intensity of the stimulus, ROS cannot always induce PCD, but can enhance cell survival.

Suppression of gamma-glutamyl transpeptidase activity could reduce H_2O_2 production and disregulate PARP activity (a caspase substrate), resulting in apoptosis (DEL BELLO et al., 1999). It was also demonstrated that both degradation of H_2O_2 by CAT or inactivation of H_2O_2 by trolox diminished cell survival. IRANI et al. (1997) had revealed that Ras-expressing transformed fibroblasts (a positive mutation on NADPH-oxidase) produced large amounts of $O_2^{\bullet-}$ which could be the key factor in operating the cell survival pathways. It was also demonstrated that NAC inhibited the mitogenic activity of those Ras-transformed cells. In fact, Ras signaling could induce either apoptosis, through Raf signaling mechanisms, or cell survival, via stimulation of phosphatidylinositol-3-kinase and its protein kinase effectors (KAUFFMANN-ZEH et al., 1997).

To resolve the contradictory effects of ROS in cell living, CLÉMENT & PERVAIZ (1999) had proposed that in cell homeostasis there is a normal concentration of $O_2^{\bullet-}$., after all the normal intracellular environment is reduced (GUTTERIDGE, 1999). When these normal $O_2^{\bullet-}$ levels are affected, cells can dye. If ROS overproduction occurs, cells can die in a necrotic morphological pattern but under hypoxic conditions, characterized by lower levels of $O_2^{\bullet-}$, cells undergo apoptosis (CLÉMENT & PERVAIZ, 1999). Perhaps this explains why natural products (tea polyphenols) are effective against cancer cell lines.

THOMPSON (1998) proposed a critical discussion about cell death types and respective signals. He showed that cells could die in many different ways and that the establishment of death models can lead to some mistakes, as the same molecule can participate in different biochemical pathways linked to the cell's death or to the cell's survival.

Conclusions and directions

Partially, at least, oxidative stress and its products are associated with PCD. But if PCD is good to eliminate neoplastic cells from the body (FERRARI, 2000B), it is bad for the heart or nervous tissues, and those cannot loose cells. Due to its various FR scavenger actions and different effects on cell physiology, antioxidants must be used with caution. Once each molecule can employ different transcription pathways, involving specific membrane receptors, signaling messengers and nuclear targetings (MONTEIRO & STERN, 1995; PALMER & PAULSON, 1997), the use of antioxidants should be selective regarding a specific pathology and cannot be used in all circumstances, specially while the mechanisms of action have not yet been well clarified. Some mechanisms mediated by the oxidative stress and LO on signal transduction and apoptosis are summarized in Tables 2 and 3.

There are many cellular ways to prevent apoptosis: 1) Expression of bcl-2 and bcl-x that inhibit caspases and cytochrome c release (CLUTTON, 1997; GARDNER et al., 1997; ROSSÉ et al.,

1998); 2) Inhibition of NFkB inhibitor (IkB) and, consequently, expression of NFkB nuclear factors (PALMER & PAULSON, 1997; HERRMANN et al., 1997a); 3) Expression of heat-shock proteins and metallothioneins (SAMALI et al., 1996); 4) Production of GSH and, not always, SOD & CAT (CURI et al., 1998; YAMAMASU et al., 1997); 5) Increase of extracellular Mg^{2+} makes decrease glutamate release (ZHANG et al., 1996); 6) Increase of extracellular K^{+} promotes cytosolic and mitochodrial Ca^{2+} storage (YU et al, 1997); 7)Inhibition of the p53 nuclear factor (SAMALI et al., 1996; WYLLIE, 1997).

Use of extrinsic factors, such as iron chelators (deferroxamine), calcium chelators (EGTA, thapsigargin) and antioxidants can also provide protection against ROS-induced PCD.

Concluding, the research on the physiological role of oxidants and antioxidants in apoptosis is too important to establish the cellular and molecular basis of medical therapies, nutritional counseling and preventive medicine measures.

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References

ALCOUFFE, J., CASPAR-BAUGUIL, S., GARCIA, V., SALVAYRE, R., THOMSEN, M., BENOIST, H. 1999. J. Lipid Res. **40**: 1200-1210.

ANJUM, R. & KHAR, A. 1997. Exp. Cell Res. **236**: 371-377.

AYALA-TORRES, S., MOLLER, P.C., JOHNSON, B.H. & THOMPSON, E.B. 1997. Exp. Cell Res. **235**: 35-47.

BARCINSKI, M. 1998. Genet. Mol. Biol. **21**: 21-4.

BASERGA, R. 1995. Cancer Res. **55**: 249-252.

BUTTKE, T. M. & SANDSTROM, P. A. 1995. Free Rad. Res. **22**: 389-397.

CHAU, Y-P., SHIAH, S-G., DON, M-J. & KUO, M-L. 1998. Free Rad. Biol. Med. **24**: 660-670.

CLÉMENT, M-V. & PERVAIZ, S. 1999. Free Rad. Res. **30**: 247-252.

CLUTTON, S. 1997. Brit. Med. Bull. **53**: 662-668.

CURI, T. C. P., MELO, M. P. DE, PALANCH, A. C., MIYASAKA, C. K. & CURI, R. 1998. Cell Biochem. Func. **16**: 43-49.

DEL BELLO, B., PAOLICCHI, A., COMPORTI, M., POMPELLA, A. & MAELLARO, E. 1999. Faseb J. **13**: 69-79.

DELONG, M. 1998. Ann. NY Acad. Sci. **842**: 82-90.

DEVITT, A., MOFFATT, O. D., RAYKUNDALIA, C., CAPRA, J. D., SIMMONS, D. L. & GREGORY, C. D. 1998. Nature **392**: 505-509.

DREWES, G., EBNETH, A., PREUSS, U., MANDELKOW, E-M. & MANDELKOW, E. 1997. Cell **89**: 297-308.

DOBMEYER, T. S., FINDHAMMER, S., DOBMEYER, J. M., KLEIN, S. A., RAFFEL, B., HOELZER, D., ET AL. 1997. Free Rad. Biol. Med. **22**: 775-785.

DRAPPER, H. H., POLENSEK, L., HADLEY, M. & MCGIRR, L. G. 1984. Lipids **19**: 836-843.

DRUKARCH, B., SCHEPENS, E., STOOF, J. C., LANGEVELD, C. H. & MUISWINKEL, F. L. VAN. 1998. Free Rad. Biol. Med. **25**: 217-220.

ESCARGUEIL-BLANC, I., SALVAYRE, R. & NÉGRE-SALVAYRE, A. 1994. Faseb J. **8**: 1075-1080.

ESTERBAUER, H. 1993. Am. J. Clin. Nutr. **57**(suppl.): 779S-786S.

ESTERBAUER, H., SCHAUR, R. J. & ZOLLNER, H. 1991. Free Rad. Biol. Med. **11**: 81-128.

FANIDI, A. & EVAN, G. 1994. Trends Biotechnol. **12**: 219-221.

FERRARI, C. K. B. 1998. Rev. Nutr. **11**: 3-14.

FERRARI, C. K. B. 1999. Hig. Aliment. **13**(60): 16-20.

FERRARI, C. K. B. 2000a. Arq. Bras. Med. (accepted).

FERRARI, C. K. B. 2000b. Rev. Univ. Catol. Bras. (accepted).

FESUS, L., MADI, A., BALAJTHY, Z., NEMES, Z. & SZONDY, Z. 1996. Experientia **52**: 942-948.

FRIEDMAN-HAIMOVITZ, A., KOLESNICK, R. N. & FUKS, Z. 1997. Brit. Med. Bull. **53**: 539-553.

GAMALEY, I. A., KIRPICHNIKOVA, K. M. & KLYUBIN, I. V. 1998. Free Rad. Biol. Med. **24**: 168-174.

GARDNER, A. M., XU, F-H., FADY, C., JACOBY, F. J., DUFFEY, D. C., TU, Y. & LICHTENSTEIN, A. 1997. Free Rad. Biol. Med. **22**: 73-83.

GUTTERIDGE, J.M.C. 1999. Redox Report **4**: 129-131.

HALESTRAP, A. P., GRIFFITHS, E. J. & CONNERN, C. P. 1993. Biochem. Soc. Trans. **21**: 353-358.

HALLIWELL, B. 1995. Am. J. Clin. Nutr. **61**(suppl.): 670S-677S.

HANNUN, Y. A. 1996. Science **274**: 1855-1859.

HERRMANN, J. L., BEHAM, A. W., SARKISS, M., CHIAO, P. J., RANDS, M. T., BRUCKHEIMER, E. M., BRISBAY, S. & MCDONNELL, T. J. 1997a. Exp. Cell Res. **237**: 101-109.

HERRMANN, J. L., MENTER, D. G., BEHAM, A., ESCHENBACH, A. VON, MCDONNELL, T. J. 1997b. Exp. Cell Res. **234**: 442-451.

IRANI, K., XIA, Y., ZWEIER, J. L., SOLLOTT, S. J., DER, C. J., FEARON, E. R., SUNDARESAN, M., FINKEL, T. & GOLDSCHMIDT-CLERMONT, P. J. 1997. Science **275**: 1649-1652.

ISHII, N., FUJII, M., HARTMAN, P. S., TSUDA, M., YASUDA, K., SEMOO-MATSUDA, N., ET AL. 1998. Nature **394**: 694-696.

ISLAM, K. N., KAYANOKI, Y., KANETO, H., SUZUKI, K., ASAHI, H., FUJII, J., ET AL. 1997. Free Rad. Biol. Med. **22**: 1007-1017.

JABS, T. 1999. Biochem. Pharmacol. **57**: 231-245.

JAIN, S. K. 1984. J. Biol. Chem. **259**: 3391-3394.

JEWETT, S. L., ROCKLIN, A. M., GHANEVATI, M., ABEL, J. M. & MARACH, J. A. 1999. Free Rad. Biol. Med. **26**: 905-918.

JI, C., ROUZER, C. A., MARNETT, L. J. & PIETENPOL, J. A. 1998. Carcinogenesis **19**: 1275-1283.

JURMA, O. P., HOM, D. G. & ANDERSEN, J. K. 1997. Free Rad. Biol. Med. **23**: 1055-1066.

KAUFFMANN-ZEH, A., RODRIGUEZ-VICIANA, P., ULRICH, E., GILBERT, C., COFFER, P., DOWNWARD, J. & EVAN, G. 1997. Nature **385**: 544-548.

KIM, Y-M., DE VERA, M. E., WATKINS, S. C. & BILLIAR, T. R. 1997. J. Biol. Chem. **272**: 1402-1411.

KINSCHERF, R., CLAUS, R., WAGNER, M., GEHRKE, C., KAMENCIC, H., HOU, D., ET AL. 1998. Faseb J. **12**: 461-467.

KRISTIÁN, T & SIESJÖ, B. K. 1998. Stroke **29**: 705-718.

KRUMAN, I., BRUCE-KELLER, A. J., BREDESEN, D., WAEG, G. & MATTSON, M. P. 1997. J. Neurosci. **17**: 5089-5100.

KUBOW, S. 1992. Free Rad. Biol. Med. **12**: 63-81.

KÜÇÜK, O., STONER-PICKING, J., YACHNIN, S., GORDON, L. I., WILLIAMS, R. M., LIS, L. J., ET AL. 1994. Lipids **29**: 657-660.
LIU, X., ZOU, H., SLAUGHTER, C. & WANG, X. 1997. Cell **89**: 175-184.
MALORNI, W., STRAFACE, E., GENOVA, G. DI, FATTOROSSI, A., RIVABENE, R., CAMPONESCHI, B., ET AL. 1997. Exp. Cell Res. **236**: 436-445.
MAULIK, N., YOSHIDA, T. & DAS, D. K. 1998. Free Rad. Biol. Med. **24**: 869-875.
MEβMER, V. K. & BRÜNE, B. 1996. Arch. Biochem. Biophys. **327**: 1-10.
MCCONKEY, D. J. & ORRENIUS, S. 1994. Trends Cell Biol. **4**: 370-375.
MONTEIRO, H. P. & STERN, A. 1995. Ciênc. Cult. (J. Braz. Assoc. Adv. Sci.) **47**: 395-401.
OARADA, M., MIYAZAWA, T., FUJIMOTO, K., ITO, E., TERAO, K. & KANEDA, T. 1988. Agric. Biol. Chem. **52**:2101-2.
OLIVETTI, G., ABBI, R., QUAINI, F., KAJSTURA, J., CHENG, W., NITAHANA, J. A., ET AL. 1997. New Engl. J. Med. **336**: 1131-1141.
PALMER, H. J. & PAULSON, K. E. 1997. Nutr. Rev. **55**: 353-361.
QIU, X-B., SCHÖNTHAL, A. H. & CADENAS, E. 1998. Free Rad Biol. Med. **24**:848-54.
RAMARATHNAM, N., OSAWA, T., OCHI, H. & KAWAKISHI, S. 1995. Trends Fd. Sci. Technol. **6**: 75-82.
REID, G. G., EDWARDS, J. G., MARSHALL, G. E., SUTCLIFFE, R. G. & LEE, W. R. 1997. Exp. Cell Res. **236**: 86-93.
RICHTER, C. 1998. Environm. Health Perspect. **106**(suppl.5): 1125S-1130S.
RIZZUTTO, R., PINTON, P., CARRINGTON, W., FAY, F. S., FOGARTY, K. E., LIFSHITZ, L. M., ET AL. 1998. Science **280**: 1763-1766.
ROLLET-LABELLE, E., GRANGE, M-J., ELBIM, C., MARQUETTY, C., GOUGEROT-POCIDALO, M-A. & PASQUIER, C. 1998. Free Rad. Biol. Med. **24**: 563-572.
ROSSÉ, T., OLIVIER, R., MONNEY, L., RAGER, M., CONUS, S., FELLAY, I., ET AL. 1998. Nature **391**: 496-499.
SAMALI, A., GORMAN, A. M. & COTTER, T. G. 1996. Experientia **52**: 933-941.
SANDOVAL, M., ZHANG, X-J., LIU, X., MANNICK, E. E., CLARK, D. A. & MILLER, M. J. S. 1997. Free Rad. Biol. Med. **22**: 489-495.
SATA, M. & WALSH, K. 1998. J. Clin. Invest. **102**: 1682-1689.
SAVILL, J. 1998. Nature **392**: 442-443.
SEVANIAN, A. & PETERSON, A. R. 1986. Fd. Chem. Toxic. **24**: 1103-1110.
STEINBERG, D. 1988. Metabolism of lipoproteins and their role in the pathogenesis of atherosclerosis. Atherosc. Rev. **18**: 1-23.
STRIDH, H., KIMLAND, M., JONES, D. P., ORRENIUS, S. & HAMPTON, M. B. 1998. FEBS Lett. **429**: 351-355.
THOMPSON, E. B. 1998. Annu. Rev. Physiol. **60**: 525-32.
WIEDAU-PAZOS, M., GOTO, J. J., RABIZADEH, S., GRALLA, E. B., ROE, J. A., LEE, M. K., VALENTINE, J. S. & BREDESEN, D. E. 1996. Science **271**: 515-518.
WU, Y-C. & HORVITZ, H. R. 1998. Nature **392**: 501-504.
WYLLIE, A. H. 1997. Brit. Med. Bull. **53**: 451-465.
YAMAMASU, S., SATO, E. F., OGITA, S. & INOUE, M. 1997. Free Rad. Biol. Med. **23**: 100-109.
YANG, G-Y., LIAO, J., KIM, K., YURKOW, E. J. & YANG, C. S. 1998. Carcinogenesis **19**: 611-616.
YIN, Y., TERAUCHI, Y., SOLOMON, G. G., AIZAWA, S., RANGARAJAN, P. N., YASAKI, Y., ET AL. 1998. Nature **391**: 707-710.
YU, S. P., YEH, C-H., SENSI, S. L., GWAG, B. J., CANZONIERO, L. M. T., FARHANGRAZI, Z. S., ET AL. 1997. Science **278**: 114-117.
ZHANG, L., RZIGALINSKI, B. A., ELLIS, E. F. & SATUN, L. S. 1996. Science **274**: 1921-1923.
ZHIVOTOVSKY, B., ORRENIUS, S., BRUSTUGUM, O. T. & DφSKELAND, S. O. 1998. Nature **391**: 449-450.
ZHOU, Y-P., TENG, D., DRALYUK, F., OSTREGA, D., ROE, M. W., PHILIPSON, L., POLONSKY, K. S. 1998. J. Clin. Invest. **101**: 1623-1632.
ZIMMERMAN, G. A., PRESCOTT, S. M. & MCINTYRE, T. M. 1995. J. Nutr. **125**(suppl.): 1661S-1665S.

Table 1. Cellular and biochemical characteristics of apoptosis.

Membrane features:

- Membrane blebbing
- Loss of membrane specialization structures (desmosomes and microvilli)
- Loss of cell to cell adhesion and loss of other cell contacts
- Formation of membrane-enclosed vesicles: the apoptotic bodies
- Expression of Tumor Necrosis Factor Receptors, such as FAS (Apo-1/CD95) and cysteine-rich receptors [nerve growth factor receptor and vitronectin receptor (CD36)]
- Exposition of phosphatidylserine to the external environment (outside)
- Changes in the carbohydrates` composition of plasma membrane
- Activation of membrane-born signal transduction pathways

Cytoplasmic characteristics:

- Cytoplasmic condensation
- Compaction of cytoplasmic organelles
- Endoplasmic reticulum budding
- Changes in membrane $\Leftrightarrow$ cytosol $\Leftrightarrow$ mitochondrial influx of Ca^{2+}, K^{+}, etc.
- Requirement of ATP to the synthesis of mRNA and proteins, and to remove the water content of cytoplasm
- Activation of Ca^{2+}-dependent transglutaminases $\Rightarrow$ $\varepsilon(\gamma$-glutamyl)lysine linkages between polypeptide chains $\Rightarrow$ protein polymerization

Nuclear features:

- Condensation and margination of nuclear chromatin
- Activation of Ca^{2+}-Mg^{2+}-endonuclases $\Rightarrow$ internucleosomal DNA fragmentation into 50 and 180-200bp oligomers
- Production of caspases $\Rightarrow$ cleavage of nuclear and cytoplasmic proteins $\Rightarrow$ loss of the integrity of cellular structures

Table 2. Apoptotic pathways induced by oxidative stress.

Reactive oxygen species ⇒ depletion of antioxidants (cofactors and repair enzymes) ⇒ Apoptosis
Reactive oxygen species ⇒ increasing of mitochondrial Ca^{2+} influx ⇒ activation of Ca^{2+}-dependent transglutaminases and endonucleases ⇒ Apoptosis
Reactive oxygen species ⇒ Pore transition oppening ⇒ decrease of mitochondrial transmembrane potential ⇒ production of more Reactive oxygen species during ATP synthesis ⇒ releasing of Ca^{2+} and depletion of NAD(P)H and glutathione ⇒ amplification of oxidative stress ⇒ Apoptosis or necrosis
Increased Ca^{2+} influx through the mitochondria ⇒ damage and leakage of mitochondrial content ⇒ cytochrome c release ⇒ caspase-9 ⇒ Apoptosis
Tea polyphenols, quinones, β-lapachone, and anticancer vegetable molecules from cruciferous, apples and garlic ⇒ increasing of H_2O_2 production⇒ Apoptosis

Table 3. Apoptotic pathways induced by lipid peroxidation products.

Reactive oxygen species ⇒ breakdown of membrane unsaturated lipids ⇒ release of arachidonates, diacylglicerols and ceramides ⇒ Apoptosis or Inflamation
Sphingomyelin hydrolysis through sphingomyelinases in biological membranes ⇒ Ceramides ⇒ activation of Rac-1 surface protein ⇒ mitogen-activated protein kinase (MAPK) signaling pathways (MEKK1/SEK1) ⇒ activation of small-activated protein kinases (SAPK/JUNK) ⇒ activation of the ICE proteases ⇒ execution of Apoptosis
Ceramides ⇒ mitochondrial injury ⇒ releasing of cytochrome c ⇒ Apoptosis
Reactive oxygen species, Tumor necrosis factor, UV, Transforming growth factor-β1, hydroperoxides and lipid oxidation products ⇒ induction of MAPK signaling pathways (MEKK1/MEKK3 activate JUNK/p38 that induce cJUN/ATF2 proteins) ⇒ Activator protein-1 (AP-1) ⇒ Apoptosis
Oxidative modified LDL ⇒ induction of p53 and Mn-SOD associated with depletion of GSH ⇒ Apoptosis
Oxidative modified LDL ⇒ expression of Tumor necrosis factor receptor family (Fas and its ligand Fas-L) ⇒ Apoptosis
Hydroxynonenal can induce apoptosis