

Ricin-Induced Toxicity: The Role of Oxidative Stress

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ABSTRACT

Ricin belongs to the type II ribosome inactivating proteins which are heterodimeric glycoproteins that contain a toxophoric A-chain and a lectin B-chain joined together by a disulfide bond. Worldwide, one million tons of castor beans (*Ricinus communis*) are processed annually in the production of castor oil; the waste mash from this process is five percent ricin by weight. Ricin has a history of use as a weapon of war, terror, and assassination, it can be prepared as liquid, crystals, or dry powder and it can be disseminated as an aerosol, injected into a victim, or used to contaminate food or water on a small scale. The toxicity of ricin varies according to the route of administration, but the clinical symptoms frequently are related to a severe inflammatory response and multiorgan failure. The cytotoxicity of ricin is commonly attributed to the inhibition of protein synthesis consequent to ribosomal damage. Several studies have also shown that administration of ricin to animals or exposure of cellular systems to ricin result in biochemical, cellular, and functional disturbances consistent with the occurrence of oxidative stress. The ability of antioxidants to ameliorate ricin-induced biochemical and cellular alterations also implicates oxidative stress as a possible contributing mechanism in ricin toxicity. In this review, the role of oxidative stress in ricin-induced cytotoxicity will be discussed.

INTRODUCTION

Ricin belongs to the type II ribosome inactivating proteins which are heterodimeric glycoproteins that contain a toxophoric A-chain and a lectin B-chain joined together by a disulfide bond (Endo, 1987a, b; Olsnes, 2004; Stirpe, 2004). The A-chain is transferred across the cell membrane by the B-chain via endocytotic vesicles into cells (Lord, 1998; Sandvig, 2002; Hartley, 2004; Roberts, 2004). The B chain binds to cell surface carbohydrates containing galactose or *N*-acetylgalactosamine residue which then allows the A-chain to enter the cell. Once inside the cell, the A-chain undergoes retrograde transport by receptor-mediated endocytosis, resulting in the toxin being transported through the Golgi complex into the cytosol after the reduction of the disulphide bond. Once in the cytosol, the A chain exhibits RNA N-glycosidase activity and inactivates ribosomes by enzymatically removing a specific adenine residue from the 28S RNA of the 60S ribosomal subunit. The adenine residue removed by ricin action is crucial for the binding of elongation factors. A consequence of the ricin-induced depurination is the cessation of protein synthesis (Olsnes, 1972; Endo, 1987a, b; Lord, 1998; Sandvig, 2002; Hartley, 2004; Roberts, 2004). The clinical latency between exposure and symptoms, ranging between 8 to 24 hours, has been attributed to the transport of the ricin to the interior of the cell.

The exact cause of ricin-induced cell death is unknown but results from several studies have shown that, regardless of the route of administration, ricin A-chain cause organ and tissue lesions that might be the result of vascular disturbances induced by the toxin rather than a direct effect of the toxin itself (Franz, 1997; Howat, 1988). Clinical trials have shown that administration of ricin A-chain immunotoxin caused vascular syndrome characterized by hypoalbuminemia and edema (Soler-Rodriguez, 1993; Schnell, 2003). In humans, the estimated lethal dose of ricin is 1 to 10 μg per kg body weight following inhalation or injection (Smallshaw, 2002). In mice, rats, and primates, high doses of ricin via inhalation produces severe enough pulmonary damage to cause death probably due to hypoxemia resulting from massive pulmonary edema and alveolar flooding (Wilhelmsen, 1996; DaSilva, 2003; Roy, 2003). In rats, inhaled ricin has an LD₅₀ of 3.7 $\mu\text{g}/\text{kg}$ and is associated with pulmonary edema, acute destructive alveolitis and necrosis/apoptosis of the lower respiratory tract epithelium (Gareth, 1995). Rats treated with ricin at a dose of 10 $\mu\text{g}/\text{kg}$ body weight (intravenous administration- iv) showed no alterations in hepatic protein synthesis but treatment adversely affected the sinusoidal cells in the liver consequently resulting in hepatocyte necrosis (Derenzini, 1976). Intravenous administration of ricin to mice (120 $\mu\text{g}/\text{kg}$ body weight) resulted in hemolytic uremic syndrome, including thrombotic microangiopathy, hemolytic anemia, and acute renal failure (Korcheva, 2005; Fu, 2004). In vitro studies with human umbilical vein endothelial cells showed that ricin damages endothelial cells (Baluna, 2000). The cytotoxicity of ricin and other ribosome-inactivating proteins are commonly attributed to the inhibition of protein synthesis consequent to ribosomal damage. A lesser known, but nevertheless important pathophysiologic event in ricin-induced toxicity appears to be oxidative stress (Schulze-Osthoff, 1992; 1993). Oxidative stress can be defined as a disturbance in the prooxidant-antioxidant balance in favor of the prooxidant, leading to damage.

Oxidative stress can be caused by an elevation in the steady-state concentration of reactive oxygen species (ROS), reactive nitrosative species (RNS) and reactive chlorine species (RCS). A large number of drugs and other xenobiotics can stimulate the generation of reactive oxygen species by redox cycling (Sies, 1987; Comporti, 1989; Stohs, 1995; Suntres, 2002). Cellular enzymatic processes, either in the normal metabolic function (electron transport in the mitochondria, microsomes) or disease states (xanthine oxidase in ischemia-reperfusion, cytochrome P-450-dependent activation of xenobiotics) are also responsible for the generation of reactive oxygen species (Sies, 1987; Fang, 2002; Djordjevic, 2004). Release of reactive oxygen species have also been demonstrated in the respiratory burst of neutrophils and macrophages (Forman and Torres, 2001; Di Virgilio, 2004). ROS, RNS and RCS indiscriminantly react with any cellular structure (Sies, 1987; Toyokuni, 1999; Forman, 2001; Fang, 2002; Di Virgilio, 2004; Djordjevic, 2004) and regulate a broad array of signal transduction pathways (Forman, 2002; Virgilio, 2004; Djordjevic, 2004). Several transcription factors such as AP-1, NFkB, MAF, NRL and NF-IL6 are regulated by the intracellular redox state of cells, a status that determines the magnitude of oxidative stress (Kamata, 1999; Hsu, 1994; Xanthoudakis, 1994; Kerrpola, 1994; Jang, 2004; Tas, 2005).

Oxidative stress can also trigger apoptosis or programmed cell death (Forman, 2002; Djordjevic, 2004; Goldenthal, 2004; Kadenbach, 2004). Activated neutrophils responding to inflammatory stimulation produce reactive oxygen species which are known to attack endothelial cells lining the vascular wall and trigger apoptosis. Endothelial cells also produce reactive oxygen species inside the cell that can contribute to oxidative stress and apoptosis, such as during ischemia/reperfusion injury (Djordjevic, 2004; Kadenbach, 2004). Oxidants can also induce apoptosis by changing cellular redox potentials by altering the reduced glutathione to oxidized glutathione (GSH/ GSSH) as well as by decreasing reducing equivalents, such as NADH and NADPH in the mitochondria (Zoratti, 1995; Bernardi, 1996; Bernardi and Petronilli, 1996). A change in the redox state, an effect also observed following administration of several toxins, facilitates the formation of permeability transition pores (PT pores), leading to the subsequent release of cytochrome c (Dalton, 1999). Mitochondrial release of cytochrome c serves as the key regulator of apoptosis because once it is released from the intermembrane space the cell is irreversibly committed to death (Orrenius, 2004).

Maintenance of cell integrity depends on the balance between cellular activation and antioxidant defense systems. Imbalances may occur due to: i) increased generation of reactive oxygen species overwhelms the defense system; ii) the defense system is severely compromised and incapable of detoxifying the normal flux or reactive metabolites; and iii) a combination of increased production and decreased detoxication occurs. The antioxidant defense system consists of enzymatic or non-enzymatic antioxidants.

The major antioxidant enzymes found in cells are superoxide dismutase, catalase and glutathione peroxidase. Three different types of SOD have been isolated and characterized: a copper and zinc containing form (Cu-Zn-SOD) that is localized in the

cytosol; a manganese-containing SOD (MnSOD) localized in the mitochondria; and, an extracellular form (EC-SOD) in the extracellular matrix. Catalase, which catalyzes the detoxication of hydrogen peroxide to water and oxygen, is localized mainly in peroxisomes, although some activity has been detected in mitochondria and cell cytoplasm. Glutathione peroxidase, plays a major role in the detoxication of hydrogen peroxide and other hydroperoxides and lipid peroxides via the glutathione redox cycle (Sies, 1987; Gutteridge, 2000).

The non-enzymatic antioxidants include vitamin C (ascorbic acid), urate, vitamin E, beta-carotene and other micronutrients such as carotenoids, polyphenols and selenium. Vitamin E, the principal lipid-soluble antioxidant in the body, is composed of four tocopherols and four tocotrienols and due to its lipophilicity it is present in all cellular membranes (Chow, 2004). Vitamin E neutralizes the highly reactive singlet oxygen molecules and protects polyunsaturated fatty acids in cell membranes from peroxidation (Buettner, 1993). Ascorbic acid, a water-soluble vitamin, is effective in scavenging free radicals, including hydroxyl radical, aqueous peroxy radicals, and superoxide anion and is considered to be one of the most important antioxidants in extra cellular fluids (Halliwell, 1996; Carr, 1999; Evans, 2001). Glutathione (GSH) is the most abundant non-protein thiol in living organisms and plays a crucial role in intracellular protection against toxic compounds, such as reactive oxygen species and other free radicals (Anderson, 1997; Anderson, 1998). It can function as a nucleophile to form conjugates with many xenobiotic compounds and/or their metabolites and can also serve as a reductant in the metabolism of hydrogen peroxide, as well as other organic hydroperoxides. Also, glutathione is important as a redox buffer (GSH/GSSH) by playing a critical role in the regulation of redox regulated transcription factors (e.g. NFkB) (Schafer, 2001).

RICIN-INDUCED TISSUE TOXICITY.

Ricin is quite stable and extremely toxic to the cells of different organs such as the liver, kidney, lung, pancreas, intestine, and thyroid (Sadani, 1997; Franz, 1997; Greenfield, 2002; DaSilva, 2003). This toxicity is largely dependent on the route of ricin exposure, an effect most likely attributed to the lectin properties of ricin. As indicated previously, the chain B of ricin contains lectin which recognizes and binds to galactosides of cell-surface carbohydrates (Sandvig, 2002; Hartley, 2004; Roberts, 2004). Lectins are being investigated as carrier molecules to target drugs specifically to different cells and tissues including the gastrointestinal tract, lungs, liver, nasal mucosa, buccal cavity and the eye (Bies, 2004). Inhalation of ricin results in pathologic changes within 8 hours. Severe respiratory distress and other symptoms are followed by acute hypoxic respiratory failure in 36-72 hours. Ingestion of ricin results in gastrointestinal hemorrhage, necrosis of the liver, spleen and kidneys; severe localized muscle pain; regional lymph node necrosis, and moderate involvement of visceral organs. Intravenously administered ricin is found in the spleen followed by kidneys, heart, liver, and thymus (Fodstad, 1976, 1979; Ramsden, 1989; Franz, 1997; Stirpe, 2004; Bismuth, 2004).

THE ROLE OF OXIDATIVE STRESS IN RICIN TOXICITY.

Studies have demonstrated that following administration of ricin to animals or exposure of cellular systems to ricin there are biochemical, cellular, and functional disturbances consistent with the occurrence of oxidative stress. Challenge of mice with ricin has resulted in hepatic lipid peroxidation, glutathione depletion, DNA single strand breaks and increased urinary excretion of carbonyl compounds (Muldoon et al., 1992, 1994; Kumar, 2003). Increases in kidney lipid peroxidation with concomitant decreases in GSH also occur. In addition decreases in plasma SOD and increases in catalase activities following exposure of animals to ricin also suggest a significant oxidative stress component (Kumar, 2003). In another study, it was demonstrated that the enzyme xanthine oxidoreductase was converted from the dehydrogenase to the oxidase form in the liver of ricin-challenged animals (Battelli, 1996). Xanthine oxidase catalyzes the oxidation of hypoxanthine to xanthine and can further catalyze the oxidation of xanthine to uric acid leading to the production of both superoxide radicals ($O_2^{\cdot-}$) and H_2O_2 . Also, it has been shown that ricin administration resulted in the destruction of thyroid follicles and a reduction in circulating thyroid hormones. The effect on the thyroid follicles and circulating hormone are attributed to the production of reactive oxygen species (Sadani, 1997). Exposure of human cervical cancer cells to ricin resulted in time-dependent increases in intracellular reactive oxygen species production with concomitant reductions in cellular GSH levels and cell viability (Rao, 2005). Muldoon *et al.*, (Muldoon, 1996) reported that challenge of mice with ricin resulted in dose-dependent increases in macrophage, microsomal, and mitochondrial superoxide anion production.

The ability of antioxidants to ameliorate ricin-induced biochemical and cellular alterations also implicates oxidative stress as a possible contributing mechanism in ricin toxicity. In a lethality study based on percentage survival and time to death following a ricin LD_{100} of 25 $\mu\text{g}/\text{kg}$ (ip), vitamin E succinate and butylated hydroxyanisole extended the survival time in response to a lethal dose of ricin (Muldoon and Stohs, 1994). Challenge of U937 human myeloid leukemia cells with ricin resulted in significant reductions in cellular glutathione levels and the antioxidant N-acetylcysteine strongly inhibited ricin-induced apoptotic cell death in U937 cells, as judged by cytotoxicity, nuclear morphological change, and DNA fragmentation (Oda, 1999). Abrin, a toxin that belongs to the type II ribosome inactivating proteins and, is similar in structure and properties to ricin, induces cells to generate reactive oxygen species and the antioxidant N-acetylcysteine and 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl can delay abrin-induced cell death (Shih, 2001).

Sadani and Nadkarni (Sadani, 1994) demonstrated that injection of animals with low doses of ricin (1.5 $\mu\text{g}/\text{kg}$, ip) is specifically toxic to Kupffer cells without affecting the parenchymal cells. The resistance of the parenchymal cells to ricin was attributed to an elevation in superoxide dismutase, catalase, and glutathione peroxidase levels in the parenchymal cells and the elevation of lipid peroxidation products in the hepatic homogenates originated mainly from damaged kupffer cells. The observation that Kupfer cells are the first target of ricin, occurring within 4 h after ricin administration, has also been confirmed by other investigators (Skilleter, 1981; Bingen, 1987; Derenzini, 1987;

Zenilman, 1988; 1989; Magnusson, 1993). Activation of macrophages is associated with increased cytokine production, an enhanced oxidative metabolism, and an increased phagocytotic activity. The high sensitivity of Kupffer cells and other macrophages to ricin has been ascribed to the ability of ricin to bind effectively to the mannose receptors present in these cells via its high-mannose oligosaccharide side chain (Skilleter, 1981; Magnusson, 1993). It is known that mannose receptors are expressed on the membranes of macrophagic type cells, including mouse peritoneal macrophages, rat Kupffer cells, mouse microglial cells, and rat bone marrow macrophages (Stahl, 1982; Simmons, 1986; Stein, 1992; Magnusson, 1993; Battelli, 2001).

Exposure of macrophages to ricin resulted in a time- and concentration-dependent production of superoxide anion and nitric oxide (Hassoun, 1999), known to form peroxynitrite and nitration of tyrosine residues (Ischiropoulos, 1998). The role of nitric oxide in the pathogenesis of glomerular thrombotic microangiopathy has been explored in a ricin-treated rat model (Williams, 2000). Ricin has shown to cause mucosal inflammation, epithelial damage and increased myoelectric activity (Sjogren, 1994; Stojadinovic, 1997). Korcheva et al. (Korcheva, 2005) showed that ricin induces a severe inflammatory response via stimulation of numerous stress-activated protein kinases and increases in mRNA that encodes for TNF- α and IL-1.

Pulmonary toxicity was correlated to ricin's effect on the alveolar macrophages. This effect was shown to be associated with the transcriptional induction of a number of cell surface inflammatory markers and chemotactic factors (i.e., CD14, ICAM-1, MCP3, and MIP1 β) which participate in the recruitment of neutrophils or activation of tissue macrophages; collectively, these disturbances lead to acute lung injury mediated by formation of oxidants and release of proteases (Xu, 1995; Lukacs, 1996; Menten, 2001; DaSilva, 2003). This is also consistent with findings from other studies where pulmonary exposure to toxins resulted in infiltration and activation of phagocytes in the lung leading to pulmonary injury via the generation of reactive oxygen species. Antioxidants antagonize the infiltration and activation of activated phagocytes acting in response to the toxins (Suntres, 2002; Suntres, 1996; Suntres, 1998; Cadenas, 2002; Bhattacharyya, 2004).

In general, macrophages and other phagocytes produce reactive oxygen species during phagocytosis or stimulation with a wide variety of agents through activation of the membrane-associated nicotinamide adenine dinucleotide phosphate reduced (NADPH) oxidase. Once activated, phagocytes infiltrate a site and cause additional damage through the release of neutral proteinase or the generation of superoxide anions (O₂⁻) via NADPH-oxidase during the respiratory burst. Superoxide gives rise to hydrogen peroxide spontaneously or via superoxide dismutase. Production of hypochlorous acid (HOCl) can occur via the interaction with myeloperoxidase. On stimulation and phagocytosis, HOCl production by the neutrophil accounts for up to 70% of the oxygen consumed, making it the major oxidative product of neutrophils (Klebanoff, 1991). HOCl reacts readily with thiols and thioethers, Fe-S centers, and nucleotides (Alison, 1976; Winterbourn, 1985; Folkes, 1995), with amines to form reactive chloramines (Thomas, 1983), and with unsaturated fatty acids to form chlorohydrins (Winterbourn, 1992).

Excessive production of reactive oxygen species, in addition to their direct toxic actions, will also exacerbate the inflammatory process, cause further tissue damage, and drive redox-regulated gene expression.

One of the mechanisms by which ricin may initiate oxidative stress-induced cellular injury is linked to the production of tumor necrosis factor- α (TNF- α), which is coded for by the transcription factor NF κ B. TNF- α is produced mainly by activated macrophages and in smaller amounts by other cell types (Vandenabeele, 1995; Bradham, 1998). Kupffer cells, the resident hepatic macrophages, are a rich source of cytokines in the liver (Boulton, 1997; Gregory, 1998) and as discussed previously, numerous studies have shown that Kupffer cells are the first target of ricin, occurring within 4 h after ricin administration (Skilleter, 1981; Magnuson, 1993). Furthermore, pretreatment of mice with anti-TNF- α antibody 2 h prior to ricin administration significantly reduced the urinary excretion of by-products of oxidative stress such as malondialdehyde, formaldehyde, and acetone and decreased hepatic lipid peroxidation and DNA single strand breaks (Muldoon et al., 1994). Results from other studies have also confirmed the role of TNF- α in ricin toxicity as evidenced by the findings where exposure of the macrophage J744A.1 cells to ricin resulted in TNF- α release and TNF- α antibody antagonized the cytotoxic effects of ricin (Hassoun, 2000). Ricin induced the release of TNF- α and interleukin-1 beta by human peripheral-blood mononuclear cells in a dose- and time-dependent manner (Licastro, 1993). More recently, it was reported that ricin induced the expression of mRNAs for TNF- α in the Caco-2 cell line, which is derived from a human colon carcinoma and has characteristics that closely resemble intestinal epithelial cells (Yamasaki, 2004). Exposure of RAW 264.7 cells to ricin strongly induced the activation of the JNK and p38 MAPK and produced a dose-dependent increase in TNF- α mRNA and TNF- α (Korcheva, 2005). Among the other protein synthesis inhibitors examined in another study, modeccin and anisomyxin, known to trigger a ribotoxic stress response similar to ricin, also induced the release of TNF- α (Higuchi, 2003; Yamasaki, 2004).

It is well established that TNF- α regulates the inflammatory processes through the induction of genes that code for several immune mediators including interleukin (IL)-1, IL-6, IL-8, macrophage inflammatory protein (MIP)-2, granulocyte-macrophage colony-stimulating factor, intracellular adhesion molecule-1, endothelial leukocyte adhesion molecule-1. These mediators are known to enhance vascular permeability, stimulate the expression of adhesion molecules on endothelial cells, and serve as leukocyte chemoattractants (chemokines). The expression of mRNAs for IL-8, monocyte chemoattractant protein, macrophage inflammatory protein (MIP)-1 α and MIP-1 β was also observed in the ricin-challenged Caco-2 cell line (Yamasaki, 2004). Similarly, the enhanced transcriptions of macrophage colony stimulating factor-1, CD14, monocyte chemoattractant protein-3, macrophage inflammatory protein-1 β and intercellular adhesion molecule-1 were also observed in the lungs of ricin-exposed animals (DaSilva, 2003). In the same study, it was also demonstrated that ricin activated the nuclear factor (NF)- κ B/rel family of transcription factors (nuclear factor of κ light chain protein enhancer in B-cells inhibitor alpha (NFKBIA), I- κ B, tumor necrosis factor alpha-induced protein (tnfaip)-3) as well as a number of inflammatory mediators,

including IL-6, serine protease inhibitor 2.2, and interferon regulatory factor-1. The findings from these studies possibly suggest that the sustained phagocytic activity and transendothelial trafficking of immune cells into the damaged lungs of ricin-challenged animals are mediated through TNF- α -regulated processes.

Various effects of TNF- α are mediated by the induction of a cellular state consistent with oxidative stress. Reactive oxygen species have been implicated in the signaling pathways initiated by TNF- α and these ROS may also function as second messengers for TNF- α mediated signaling (Hennet, 1993; Schulze-Osthoff, 1998). Exogenously added reactive oxygen species to cells reproduces many of the toxic actions of TNF- α . Cells treated with certain antioxidants or kept under anaerobic conditions were less susceptible to TNF- α -induced cytotoxicity (Schulze-Osthoff, 1992). Furthermore, ROS are critical in the regulation of transcription factors in the AP-1 and NF- κ B families which have crucial functions in proliferation, differentiation, and morphogenesis and have been shown to be activated also in the absence of protein synthesis (Buscher, 1988; Devary, 1993).

Other studies have demonstrated that the mitochondrial electron transport system plays a key role in inducing TNF- α cytotoxicity, presumably by the formation of reactive oxygen species (Hennet, 1993; Schulze-Osthoff, 1998; Moreno-Manzano, 2000). Antimycin A, a mitochondrial inhibitor that increases the generation of reactive oxygen species, potentiates the TNF- α -triggered NF- κ B activation while depletion of the mitochondrial oxidative metabolism resulted in resistance towards TNF- α cytotoxicity, as well as in inhibition of NF- κ B activation by TNF- α (Schulze-Osthoff, 1993). It has been proposed that the apoptosis-inducing activity of abrin, a type II ribosomal inhibiting protein, could be due to a decrease in the levels of anti-apoptotic factors like Bcl-2 and oxidative stress due to mitochondrial damage leading to increased reactive oxygen production (Narayanan, 2004).

The mitochondrial pathway of apoptosis due to depletion of the intracellular NAD⁺ pool is known to be a result of PARP [poly(ADP-ribose) polymerase] activation. Moderate activation of PARP facilitates the efficient repair of DNA damage induced by several stimuli, such as reactive oxygen species or ionizing radiation. Severe genotoxic stress leads to rapid energy consumption and subsequently cell death. It has been shown that ricin is capable of inducing depletion of intracellular NAD⁺ and ATP levels, following PARP activation in U937 cells (Komatsu, 2000) resulting in apoptotic cell death. Another mechanism by which ricin may induce apoptotic cell death has centered on its ability to increase caspase -3-, caspase-6- and caspase-9- activities in human leukemia U937 cells (Villa, 1997; Komatsu, 1998; Tamura, 2002). Caspases are cysteinyl aspartate-specific proteases that play a crucial role in the apoptotic signal pathway, they are sensitive to the redox status of the cell, and their activity is blocked by excessive oxidative stress (Hampton, 1998). Komatsu et al., (Komatsu, 1998) have reported that a broad inhibitor of the caspase family proteases (Z-Asp-Ch2-DCB) and the specific serine protease inhibitor, dichloroisocoumarin, blocked ricin-induced apoptosis in human leukemia U937 cells. Furthermore, it was demonstrated that ricin-induced apoptosis of U937 cells (Oda, 1999) or human cervical cancer cells (Rao, 2005) was

associated with glutathione depletion. Although the exact role of glutathione depletion in apoptosis is unclear, selective depletion of reduced glutathione in mitochondria increase reactive oxygen cell accumulation and sensitizes cells to the apoptotic effects of TNF- α (Fernandez-Checa, 1998; Pierce, 2000).

Presently, the underlying mechanism(s) responsible for the release of TNF- α by ricin are not well understood. Higuchi et al., (Higuchi, 2004) have shown that treatment of RAW264.7 cells with ricin resulted in the induction of TNF- α , with the maximum levels of TNF- α secretion being attained at a ricin concentration that caused only partial inhibition of protein synthesis. Results from a limited number of studies examining the effect of different protein synthesis inhibitors on the induction of TNF- α release, suggested that ricin and anisomycin, which commonly act on 28rRNA and resulting in a ribotoxic stress response, may trigger the multiple signal transduction pathways through the activation of the stress-activated protein kinases (SAPKs), N-terminal-c-Jun-kinases (JNK), and p38 MAP kinase (Korcheva, 2005; Laskin, 2002; Iordanov, 1997). A major question remains as to the precise linkage between ribosomal RNA damage and induction of MAP kinase signaling cascades (Iordanov, 1997; Iordanov, 1998; Laskin, 2002). It is becoming clear that the ribotoxic stress pathway is a highly specific event and ribotoxic stressors appear to be restricted to those toxicants that interact with or damage the R/S loop near the 3-end of the 28S rRNA including ricin, anisomycin, palytoxin, and ultraviolet light radiation (Iordanov, 1997; 1998; Laskin, 2002). More recently, it was shown that the IL-8 inducing activity in ricin-challenged Caco-2 cells correlates with the RNA N-glycosidase activity and not with general protein synthesis and among the RNA N-glycosidases tested, the members of the Shiga toxin are the strongest inducers of IL-8 production in Caco-2 cells (Yamasaki, 2004).

MANAGEMENT OF RICIN-INTOXICATION.

Management of ricin-intoxicated patients depends on the route of exposure. Patients with pulmonary intoxication are managed by supportive treatment for pulmonary edema. Gastrointestinal intoxication is best managed by vigorous gastric decontamination with superactivated charcoal, followed by use of cathartics such as magnesium citrate. Volume replacement of GI fluid losses is important. In percutaneous exposures, treatment would be primarily supportive (Franz, 1997).

The cell responds to ricin toxicity by activating its defensive mechanisms such as evoking inflammatory processes, altering protein trafficking, cell differentiation and turning on apoptotic signals (Day, 2001; Elson, 2001; Hu, 2001). Very little is known of the role that these or any other gene regulatory paths may play in the intoxication process initiated by ricin. However, the knowledge of such complex paths is critical for the development of effective prophylactic and therapeutic countermeasures to ricin. So far, it has been shown that the ricin induced expression of TNF- α protein in RAW 264.7 macrophages was reduced significantly following the specific inhibition of JNK, p38 MAPK, and ERK1/2 kinases known to regulate the expression of mRNAs that encode inflammatory cytokines and chemokines. In another study, the ricin-induced cytotoxicity in macrophage J744A.1 cells was antagonized by pretreatment with a TNF- α

-antibody (Hassoun, 2000). The unregulated release of TNF- α into the circulation results in circulatory dysfunction, increased endothelial permeability and inflammation of different organs.

Antioxidant supplementation may prove to be beneficial in decreasing the oxidative stress in animals and cellular systems exposed to ricin. Several studies have shown that TNF- α induces $O_2^{\cdot-}$ formation in mitochondria which dismutates to H_2O_2 by the superoxide dismutase (Mn SOD). H_2O_2 can easily cross the mitochondrial membrane and be released into the cytosol where it could induce stimulate mitogen-activated protein kinases, such as ERK1/2, p38 and JNK, and the transcription factor, nuclear factor-kappaB, known to induce the expression of mRNA of a variety of pro-inflammatory mediators including TNF- α , interleukins, adhesion molecules and enzymes, such as cyclooxygenase (COX-2) and iNOS. These pro-inflammatory mediators are implicated in the pathogenesis of several acute inflammatory disorders. Also, TNF- α released during inflammation transiently activates neutrophils and macrophages, thereby causing them to release $O_2^{\cdot-}$ as a consequence of the activation of membrane-associated NADPH oxidase (Klebanoff, 1986). Besides NADPH oxidase, another superoxide-generating enzyme thought to be induced after ricin exposure is xanthine oxidase (XO).

It is evident that antioxidants can protect both extracellularly by scavenging toxic ROS and intracellularly by interrupting lipid peroxidation within the membrane. Also, they can interfere early in inflammatory responses by blocking or modifying the signal transduction of inflammatory cytokines and ricin, thereby modulating cellular activation. Administration of vitamin E succinate and butylated hydroxyanisole extended the survival time in rats challenged with a lethal dose of ricin (Muldoon, 1994) The antioxidant N-acetylcysteine (NAC) strongly inhibited the ricin-induced apoptotic cell death in U937 cells (Oda, 1999). Vitamin E and NAC as well as other antioxidants are known to inhibit mitogen-activated protein kinases, such as ERK1/2, p38 and JNK, and the transcription factor NF- κ B (Kyaw, 2004; Zafarullah, 2003; Cadenas, 2002). Antioxidants do not exert an indiscriminate stimulating effect on the immune cell function, are effective and safe in a large range of concentrations.

SUMMARY.

The cytotoxicity of ricin is commonly attributed to the inhibition of protein synthesis in ribosomes. To-date there are no effective prophylactic and therapeutic countermeasures to ricin and management of ricin intoxication remains supportive. More recent studies provide evidence that ricin can also mediate its toxic effects via oxidative stress-mediated mechanisms resulting to apoptosis and cell death. Ricin has been shown to selectively activate phagocytes, known to generate excessive amounts of reactive oxygen species, which in addition to their direct toxic actions, also exacerbate the inflammatory process and cause further tissue damage. The reduction of inflammation has been suggested as a viable drug target for the toxic pulmonary effects of ricin.

Presently, inflammatory mediators and other gene regulatory circuits are being investigated in order to assess the role they may play in the intoxication process initiated

by ricin. The knowledge of such complex paths and their manipulation is critical for the development of effective prophylactic and therapeutic countermeasures to ricin. Certainly, antioxidant supplementation may prove to be beneficial in decreasing the oxidative stress in animals and cellular systems exposed to ricin.

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