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TOPICAL REVIEW

Role of Contrast Media on Oxidative Stress, Ca^{2+} Signaling and Apoptosis in Kidney

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Abstract Contrast media (CM)-induced nephropathy is a common cause of iatrogenic acute renal failure. The aim of the present review was to discuss the mechanisms and risk factors of CM, to summarize the controlled studies evaluating measures for prevention and to conclude with evidence-based strategies for prevention. A review of the relevant literature and results from recent clinical studies as well as critical analyses of published systematic reviews used MEDLINE and the Science Citation Index. The cytotoxicity induced by CM leads to apoptosis and death of endothelial and tubular cells and may be initiated by cell membrane damage together with reactive oxygen species (ROS) and inflammation. Cell damage may be aggravated by factors such as tissue hypoxia, properties of individual CM such as ionic strength, high osmolarity and/or viscosity. Clinical studies indeed support this possibility, suggesting a protective effect of ROS scavenging with the administration of *N*-acetylcysteine, ascorbic acid erdosteine, glutathione and bicarbonate infusion. The interaction between extracellular Ca^{2+}, which plays a central role in intercellular contacts and production of ROS, and the in vitro toxicity of CM was also reviewed. The current review addresses the role of oxidative stress in the pathogenesis of CM in the kidney as well as current and potential novel treatment modalities for the prevention of neutrophil activation and CM-induced kidney degeneration in patients. ROS production through CM-induced renal hypoxia may exert direct tubular and vascular endothelial injury. Preventive strategies via antioxidant supplementation include inhibition of ROS generation or scavenging.

Keywords Apoptosis · Radiocontrast media · Calcium ion · Oxidative stress · Mitochondria

Abbreviations

CAT	Catalase
CM	Contrast media
GSH-Px	Glutathione peroxidase
MDA	Malonyl dialdehyde
NAC	N-acetyl cysteine
ROS	Reactive oxygen substances
SOD	Superoxidedismutase
XO	Xanthine oxidase

Introduction

The human body is equipped with a complete arsenal of defenses against external and internal aggressions. Those against the so-called reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical and hydrogen peroxide are crucial in inflammatory responses, where they participate in physiological processes such as the arachidonic acid cascade and phagocytosis (Kovacic and Somanathan 2008). ROS concentrations are kept under strict control by the activity of a complex defense system including enzymes and nonenzymatic antioxidants. These enzymatic and nonenzymatic antioxidants are also essential for inhibition of phagocytic activity related to ROS production (Nazıroglu 2009).

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Table 1 Effects of ionic radiocontrast media on antioxidants in kidney

Radicontrast	Effects	Cell/tissue	Antioxidants	References
Diatrizalo	Cell injury	Madin–Darby canine kidney	–	Schick et al. (2002)
Ioxaglate	Cell injury	Madin–Darby canine kidney	–	Schick et al. (2002)
Loversol	Oxidant/ nephrotoxic/apoptotic	Mouse proximal tubule segments and cultured proximal tubule	NAC, GSH, SOD and CAT/inhibitor	Zager et al. (2003)
Meglumin diatrizoate/iodine	Oxidant	Rat kidney	Erdosteine/MDA, SOD, CAT, GSH, histology	Yesildağ et al. (2009)
Amidotrizoate	Oxidant	Rat kidney	Erdosteine/TAS, TOS, DNA damage	Yesilyurt et al. (2011)
Iodine	Oxidant	Rat kidney	MDA, CAT, SOD, XO, GSH-Px	Cetin et al. (2008)

CAT catalase, *GSH-Px* glutathione peroxidase, *NAC* *N*-acetyl cysteine, *SOD* superoxide dismutase, *XO* xanthine oxidase, *MDA* malonyl dialdehyde.

Table 2 Effects of nonionic radiocontrast media on oxidative stress, antioxidants and apoptosis in kidney

Radicontrast	Effects	Sample	References
Iohexol	No effect	Madin–Darby canine kidney	Schick et al. (2002)
Iodixanol	No effect	Madin–Darby canine Kidney	Schick et al. (2002)
Ioxaglate(s)	Oxidant	Renal cortex of water-deprived rats	Yoshioka et al. (1992)
Iohexol	Oxidant	Renal cortex of water-deprived rats	Yoshioka et al. (1992)
Iohexol and iotrolan	Apoptotic	Human neutrophils	Fanning et al. (2002)
Hydration with normal saline	Protective	Human serum	Spargias et al. (2004)
Diatrizoic and iothalamic acids	Protective	Renal cortical slices	Harmon et al. (2009)
Diatrizoate	Protective on tubular necrosis and oxidative stress	Rat kidney	Toprak et al. (2008)

Oxidative stress is considered to be involved in the development of radiocontrast nephropathy because antioxidant-mediated protection of renal function has been demonstrated in vivo (Pannu et al. 2004). However, there is also a controversial conclusion that contrast media toxicity can be dissociated from tubular cell oxidative stress since contrast media do not increase tubular malondialdehyde (MDA) content (Zager et al. 2003). But, to our knowledge, there is little experimental evidence of ROS formation at the tubular cell level directly. For example, Xiong et al. (2006) observed increased intracellular ROS formation in renal tubular cells exposed to ioversol for 1 h. They suggested that ioversol induces ROS accumulation in renal tubular cells as early as 1 h (Xiong et al. 2006) (Table 1).

To maintain stable numbers of cells, cell division must be dynamically counterbalanced by apoptosis, a fundamental biological process involving selective cell deletion to regulate tissue homoeostasis (Tadros et al. 2008). Apoptosis occurs in the cytosol area, where it maintains a critical balance between numbers of newly divided and surviving cells (Marasa et al. 2008). Contrast media is extensively used in diagnostic imaging to change the X-ray absorption of tissues; iodinated contrast media should be biologically inert. However, there is an increasing recognition that these agents can alter cell function and viability. Recent reports suggest that iodinated contrast media can induce apoptosis in a variety of nonimmune cells including cardiac myocytes, renal tubular cells and glomerular cells (Heyman et al. 2010) (Table 2).

Radiocontrast nephropathy is a leading cause of acute renal failure (Parfrey et al. 1989). However, its pathogenesis remains poorly defined. Two dominant injury pathways have been widely discussed (Zager et al. 2003).

1. Radiocontrast media injection induces renal vasoconstriction (Tumlin et al. 2002), a process that is thought to arise from an imbalance between endothelium-derived vasoconstrictive and vasodilatory factors (e.g., endothelin, nitric oxide and adenosine) (Huber et al. 2001). If severe vasoconstriction is induced, ischemic tubular injury, culminating in cell detachment, apoptosis or necrosis, can each result (Sheridan and Bonventre 2001). Proximal tubules and medullary thick ascending limbs (Beeri et al. 1995) are particularly vulnerable to these events.
2. Following tissue ischemia, "reperfusion injury" may occur. This may arise from a number of factors, such as oxidative

stress (Sheridan and Bonventre 2001) or "realkalinization injury" induced by a rapid correction of ischemia-induced reductions in tissue pH (Zager et al. 2003).

In the last few years, a number of clinical studies have suggested that *N*-acetylcysteine may prevent contrast-induced nephropathy (Romano et al. 2008). Two additional antioxidant strategies have also aroused considerable interest: sodium bicarbonate and ascorbic acid (Romano et al. 2008).

This review suggests that the role of antioxidants such as oral *N*-acetylcysteine and ascorbic acid in the prevention of radiocontrast-induced nephropathy has yet to be defined. The literature as it currently exists is profoundly heterogeneous, making any single summary estimate invalid. Thus, the analysis could not demonstrate an added benefit of oral *N*-acetylcysteine among all individuals with preexistent renal insufficiency. Meta-regression analysis identified some important study and patient characteristics that may partially explain the heterogeneity: time of *N*-acetylcysteine administration, advanced age, presence of diabetes mellitus and volume and type of radiocontrast medium. It is biologically plausible that these characteristics would affect the relationship of *N*-acetylcysteine and, therefore, may serve to guide future research in this field (Kshirsagar et al. 2004) (Fig 1).

Radiocontrast Media and Apoptosis

Apoptosis has multiple pathways that differ according to tissue type and pathological condition. These pathways have been identified and broadly classified into two main types: extrinsic and intrinsic (Tadros et al. 2008). The extrinsic pathways include death receptor– and survival factor–withdrawal pathways. The first is activated by stimulation of certain membranous receptors like TNF-α and Fas receptors, while the latter involves activation of c-Jun and JNK, by ROS, inflammatory cytokines, mixed-lineage kinases, radiation or excitotoxicity. Both pathways subsequently activate certain cascades of factors that ultimately lead to cell death through their effect on mitochondrial membrane stability and activation of caspases (Mathai et al. 2005). Intrinsic apoptotic pathways are caused by either DNA damage or stress to the endoplasmic reticulum. DNA damage causes release of p53, which leads to mitochondrial membrane dysfunction, while endoplasmic reticulum stress causes Ca^{2+} accumulation and calpain activation, which can lead to either activation of caspases or lysosome rupture, cathepsin release or PARP-1 cleavage and ultimately DNA damage (Mathai et al. 2005; Tadros et al. 2008). In addition, Ca^{2+} may activate c-Jun and JNK pathways and start the extrinsic survival factor–withdrawal apoptotic pathway. In both pathways, cytochrome *c* is released with activation of downstream caspases and cell death. Some exceptions for which apoptosis does not require caspase activation include the release of factors like endo G and apoptosis-inducing factor from the mitochondria. These factors can bypass caspase activation and cause cellular damage and apoptosis (Yasuhara et al. 2007; Tadros et al. 2008).

Under normal circumstances, cytochrome *c* is associated with the inner mitochondrial membrane. However with

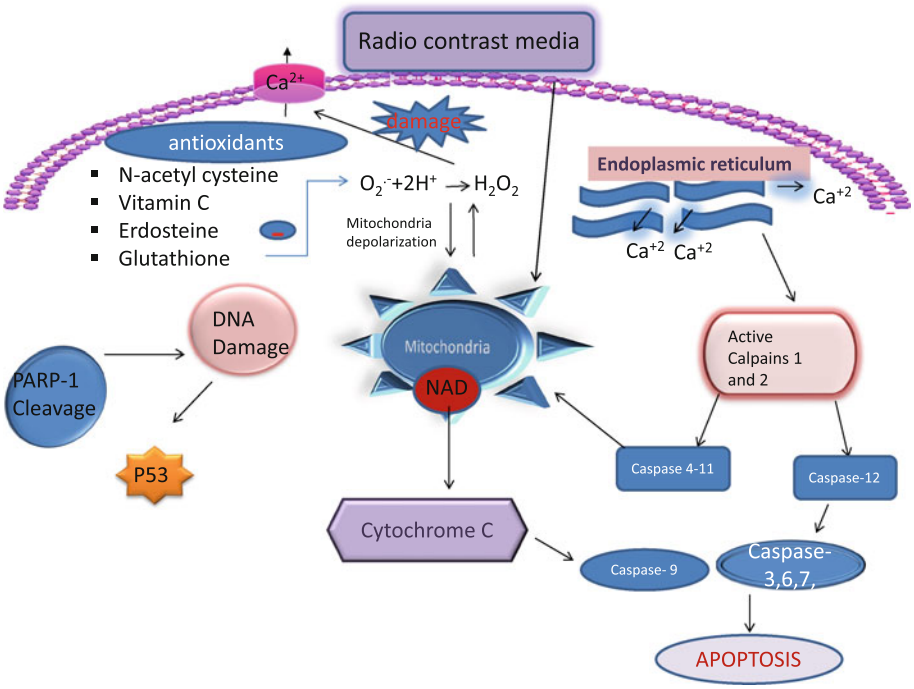

Fig. 1 Intrinsic apoptotic pathways are caused either by DNA damage or by stress to the endoplasmic reticulum. DNA damage causes release of p53, which leads to mitochondrial membrane dysfunction, while endoplasmic reticulum stress causes Ca^{2+} accumulation and calpain activation, which can lead either to activation of caspases or to lysosome rupture, cathepsin release or PARP-1 cleavage and ultimately DNA damage. Oxidative DNA damage in renal tubular cells can lead to necrosis or apoptosis of these cells and can therefore be responsible for radiocontrast-induced nephropathy, although antioxidants such as *N*-acetylcysteine, ascorbic acid, erdosteine and glutathione induce protective effects on the process

mitochondrial injury, it reaches the cytosol, where it can initiate caspase activation and ultimately cell death. With radiocontrast media addition dramatic cytochrome *c* loss into the cell supernatant solution was observed in the absence of increased lactate dehydrogenase release (Zager et al. 2003). It is notable that sublethal doses of pancreatic phospholipase A_2, which primarily attacks the plasma membrane and secondarily depresses mitochondrial function (Zager et al. 1996), also caused cytochrome *c* release. This suggests that radiocontrast medium-induced mitochondrial damage, as reflected by cytochrome *c* leak, is a downstream result of plasma membrane damage. That neither phospholipase A_2 nor radiocontrast media caused lethal cell injury in these experiments indicates that the cytochrome *c* loss into the tubule media reflected a prelethal tubular cell event. The link between plasma membrane injury and secondary mitochondrial damage and the ultimate role of cytochrome *c* release in the expression of radiocontrast media toxicity will each require substantial additional investigations (Zager et al. 1996).

Radiocontrast Media, Osmolality and Ca^{2+}

Acute renal failure is an important complication of the intravascular administration of radiocontrast agents. The major risk factor for renal injury is renal dysfunction prior to contrast media exposure. In addition to alterations leading to renal medullary hypoxia, direct cytotoxic effects of radiocontrast agents on renal tubular cells have been implicated in the pathogenesis of acute renal failure (Andersen et al. 2003).

Four classes of water-soluble radiocontrast agents are in clinical use, with distinct physicochemical properties; ionic radiocontrast agents generally have a higher osmolality than nonionic compounds as they dissociate in aqueous solutions. Since biological membranes are largely impermeable for radiocontrast agents, high-osmolar compounds are hypertonic. The osmolality/hypertonicity of radiocontrast agents could be reduced by dimerization. Whereas the only ionic dimeric radiocontrast compound, ioxaglate, is still moderately hypertonic, nonionic dimers like iodixanol are virtually isotonic with blood (Schick et al. 2002).

Direct cytotoxic effects of radiocontrast agents have been implicated in radiocontrast nephropathy. The interaction between extracellular Ca^{2+}, which plays a central role in intercellular contacts, and the in vitro toxicity of contrast media was tested in Madin–Darby canine kidney cell monolayers grown on permeable supports. Schick et al. (2002) reported that the ionic radiocontrast agents diatrizoate and ioxaglete, but not the nonionic compounds iohexol and iodixanol, reduced extracellular Ca^{2+} in vitro. However, this reduction of Ca^{2+} does not explain their cytotoxic effects, which could contribute to the pathogenesis of Madin–Darby canine kidney cells in vivo by opening intercellular junctions.

Several magnetic resonance contrast media have been shown to be cytotoxic and to trigger apoptosis, the suicidal death of nucleated cells (Elmståhl et al. 2008). Erythrocytes are similarly able to enter suicidal death, i.e., eryptosis (Lang et al. 2008). During eryptosis, erythrocytes expose phosphatidylserine at their surface (Lang et al. 2008). Phosphatidylserine exposure results from phospholipid scrambling of the cell membrane (Dekkers et al. 2002). It is elicited by an increase in cytosolic Ca^{2+} activity (Lang et al. 2008). Phosphatidylserine-exposed erythrocytes are phagocytosed and thus rapidly eliminated from circulating blood (Kempe et al. 2006). Cytosolic $[Ca^{2+}]_i$ concentration is increased, and thus, eryptosis is elicited by iso-osmotic cell shrinkage (chloride replacement by gluconate), oxidative stress (Haag-Weber and Hörl 1994), prostaglandin E_2 (Kaestner et al. 2004) and energy depletion (Klarl et al. 2006). All those challenges are at least partially effective because of the activation of Ca^{2+}-permeable cation channels (Haag-Weber and Hörl 1994). In addition to its effect on cell membrane scrambling, Ca^{2+} activates Ca^{2+}-sensitive K^+ channels (Brugnara et al. 1993) and therefore triggers the exit of KCl with osmotically obliged water, thus leading to cell shrinkage. The shrinkage then contributes to the stimulation of cell membrane scrambling (Lang et al. 2008).

Nebivolol is a β_1-adrenergic receptor antagonist with vasodilator and antioxidant properties (Veverka et al. 2006). Toprak et al. (2008) reported a protective role of nebivolol on oxidative stress. Proteinuria affects humans and other animals following use of contrast agents (Nicot et al. 1984; Toprak et al. 2008).

Radiocontrast Media and Neutrophil Function

Neutrophils are the most abundant leukocytes in the blood circulation. These cells are exposed to high concentrations of iodinated contrast media following intravascular injection. Neutrophils constitutively undergo apoptosis, and regulation of this process is critical in controlling the duration of the host response to injury and infection (Bréchard and Tschirhart, 2008). Mature neutrophils have a short life span of 8–20 h, but this can be increased severalfold if they are recruited into inflamed tissues in vivo or exposed to proinflammatory mediators, such as lipopolysaccharide, in vitro (Savill 1997). Delayed expression of neutrophil apoptosis is associated with systemic proinflammatory syndromes, such as acute respiratory distress syndrome (Fanning et al. 1999). Accelerated expression of neutrophil apoptosis is associated with an increased susceptibility to sepsis (Kusaba et al. 1998). One report suggests that iodinated contrast media increase the incidence of both local and systemic septic

complications in patients with mild acute pancreatitis (Carmona-Sánchez et al. 2000). Successful resolution of inflammation requires a balance between pro- and antiapoptotic intracellular signaling pathways in neutrophils. Any agent that alters the rate of constitutive and inflammatory neutrophil cell death could therefore have a significant deleterious effect on host immune defense and resolution of an inflammatory response (Fanning et al. 2002).

Iodinated ionic and nonionic contrast media influence cellular functions such as secretion of leukotrienes, activation of phagocytic cells and oxidative stress (Ayub and Hallett 2004; Bréchard and Tschirhart 2008). Polymorphonuclear leukocytes are primarily or secondarily involved in the pathogenesis of contrast media-induced toxicity (Böhm et al. 2008; Kayan et al. 2012). They are key players in the inflammatory process during which they are exposed to a variety of agonists that signal mostly through heterotrimeric, G protein-coupled receptors. An increase in intracellular $[Ca^{2+}]_i$ concentration is an important step within the multitude of serial or parallel signaling events that participate in the activation of neutrophil reactions such as chemotaxis, release of ROS and apoptosis (Sahin et al. 2011; Korkmaz et al. 2011). The release of superoxide radicals by NADPH oxidase that follows stimulation of the formyl peptide receptor requires an increase in $[Ca^{2+}]_i$ (Sahin et al. 2011). Recently we observed that non ionic contrast media stimulated phagocytic activity in human neutrophils (Kayan et al. 2012) although its physiological mechanisms are not clear. Contrast media may increase ROS production in kidney and polymorphonuclear leukocytes via stimulation of Ca^{2+} influx (Yoshioka et al. 1992). Fanning et al. (1999) reported also that urinary IL-18 or neutrophil gelatinase-associated lipocalin could be early biomarkers of contrast-induced nephropathy.

Iodinated ionic and nonionic contrast media have been shown to influence cellular functions such as de novo synthesis of leukotrienes, cellular adherence, locomotion and phagocytosis (Böhm et al. 2008). The effects of iodinated contrast media on blood and endothelium include controlling infections and clearing foreign material as well as necrotic and apoptotic cells; scarce reports have been published so far that present data concerning contrast media-induced modulation of phagocytosis (Farrow et al. 1994; Kayan et al. 2012). Of these, only two describe the influence of contrast media on the phagocytic function in vivo (Lillevang et al. 1994); both these studies analyzed the effects of iohexol and ioxaglate.

Although both monocytes/macrophages and neutrophils are professional phagocytes, they preferentially clear different materials (Amulic et al. 2012). For example, engulfment of apoptotic cells has been shown mainly by monocytes/macrophages, whereas phagocytosis of unopsonized foreign material is mainly carried out by neutrophils. Moreover, agent-dependent (e.g., anesthetics, barbiturates) impairment of phagocytosis may be different in monocytes and neutrophils (Heller et al. 1998). Formerly, contrast media-dependent modification of phagocytosis has been analyzed only in granulocytes (Lillevang et al. 1994). Böhm et al. (2008) investigated the effect of nonionic contrast media on both phagocytic capacity and activity in patients undergoing contrast media-enhanced computer tomography, and they observed a direct relationship between neutrophil activation and contrast media exposure.

Nephrotoxicity and Contrast Media

Nephropathy secondary to iodine-based radiocontrast agent exposure is one of the most common causes of acute renal failure in hospital settings. The mechanism of toxicity of radiocontrast agents has been the subject of much research over the past 40 years, focusing on their mechanisms of toxicity, including oxidative stress, hyperosmolarity and hemodynamic effects. These investigations have used a variety of models ranging from in vivo animal models to single-cell immortalized in vitro systems. The nephrotoxicity induced by contrast media remains a serious clinical problem, and the underlying mechanism is not completely understood. Experimental and clinical investigations suggest that ROS are critical determinants of radiocontrast nephropathy and that antioxidants can prevent this damage (Haeussler et al. 2004).

Despite huge efforts, the nephrotoxicity of Radiocontrast agents remains a serious clinical problem. Radiological procedures utilizing contrast media are being employed increasingly for both diagnostic and treatment purposes. The precise physiological insult underlying contrast nephropathy is unclear and may well involve the interplay of several pathogenic factors. These may include vasoconstriction resulting in medullary ischemia (Heyman et al. 2010), direct effects on renal tubular cells and damage caused by ROS (Bakris et al. 1990). Since chronic renal failure is associated with increased oxidative stress, patients with chronic renal impairment are more susceptible to contrast media-induced nephrotoxicity. Based on the possible role of oxidative damage in the kidney following contrast media administration, it might be anticipated that treatment with an antioxidant would minimize renal dysfunction (Haeussler et al. 2004).

Oxidative Stress, Ca^{2+} Signaling, Apoptosis and Contrast Media

Radiocontrast nephropathy is a major complication after radiographic examination and is reported to be the third most

common cause of hospital-acquired acute renal failure (Goldenberg and Matetzky 2005). Risk factors for radiocontrast nephropathy include preexisting renal insufficiency, diabetes, congestive heart failure, age over 75 years, hypercholesterolemia (Lindholt 2003) and concomitant use of nonsteroidal anti-inflammatory drugs. Although numerous trials have been proposed to provide prophylactic approaches against radiocontrast nephropathy, the results remain a matter of debate. Until recently, only intravenous hydration is generally accepted to prevent radiocontrast nephropathy (Trivedi et al. 2003; Xiong et al. 2006).

As mentioned above, experimental and clinical investigations suggest that oxidative stress is critical for radiocontrast media-induced kidney toxicity. Zager et al. (2003) investigated these issues directly at the tubular cell level. They used isolated segments of mouse cultured proximal tubule cells incubated in radiocontrast medium (loversol), and they measured cellular viability and lipid peroxidation values. They concluded that contrast media toxicity can be dissociated from tubular cell oxidant stress. Alternative mechanisms may include mitochondrial injury/cytochrome *c* release and plasma membrane damage, the latter resulting in critical protein loss, as well as a marked increase in plasma membrane susceptibility to exogenous/endogenous phospholipase A_2 attack (Zager et al. 2003).

Moreover, DNA may be damaged by free radical reactions since exposure of DNA to ROS induces base hydroxylation and strand breaks, resulting in either cell injury or apoptosis (Nazıroğlu 2007). Oxidative DNA damage in renal tubular cells can lead to necrosis or apoptosis of these cells and can therefore be responsible for radiocontrast-induced nephropathy (Haeussler et al. 2004).

It has been hypothesized that alkalizing renal tubular fluid bicarbonate may reduce injury. At physiologic concentrations, bicarbonate scavenges peroxynitrite and other reactive species generated from nitric oxide (Merten et al. 2004). In the clinical setting, the higher concentration of bicarbonate in the proximal convoluted tubule may buffer the higher production of H^+ due to cellular hypoxia and facilitate Na^+ reabsorption through the electrogenic Na/HCO_3 cotransporter (Boron 2006). The in vivo study of Romano et al. (2008) does not support the former mechanism. It may be that $NaHCO_3$ facilitates Na^+ reabsorption; this would mitigate the increase in sodium delivery to the macula densa induced by contrast media, an effect that results in vasoconstriction of the afferent arteriola through tubuloglomerular feedback. Furthermore, in their in vitro model, $NAHCO_3$ did not raise the pH of the medium in comparison to contrast medium alone (Romano et al. 2008).

As it was mentioned above, the renal blood flow decreased in kidney through radio contrast exposure. The decrease in renal blood flow and direct toxic action on renal tubular cells (Lancelot et al. 1999) have been considered to be involved in the pathogenesis of radiocontrast nephropathy. Several clinical studies have demonstrated that antioxidant agents such as *N*-acetylcysteine (Marenzi et al. 2006) and ascorbic acid (Spargias et al. 2004), rather than agents that improve renal blood flow, can produce a significant effect to prevent radiocontrast nephropathy. Furthermore, an increased level of 15-isoprostane F2t, a specific marker of oxidative stress, was observed after radiographic examination in patients with renal insufficiency (Drager et al. 2004). These results have suggested that ROS-mediated renal tubular cell injury plays an important role in the development of radiocontrast nephropathy.

Radiocontrast Media and Antioxidants

N-Acetylcysteine

The pathologic mechanisms by which radiocontrast media induced these changes were not defined. However, investigations from a number of laboratories suggest that oxidative tubular stress is involved. This conclusion is supported by findings that radiocontrast medium injection can induce or exacerbate lipid peroxidation in either normal or dehydrated rats (Yoshioka et al. 1992). The fact that administration of *N*-acetylcysteine to humans may mitigate radiocontrast nephropathy further supports this view (Briguori et al. 2002). Intravenous and oral *N*-acetylcysteine prevented also contrast medium-induced nephropathy with a dose-dependent effect in patients treated with primary angioplasty and may improve hospital outcome (Marenzi et al. 2006).

Zager et al. (2003) reported that radiocontrast media-induced cytotoxicity can be dissociated from tubular cell oxidant stress. *N*-Acetylcysteine may not prevent clinical radiocontrast media-induced acute renal failure. Radiocontrast media toxicity cannot be explained by the induction of a hypertonic milieu, as previously suggested; rather, direct compound-related toxicity appears to play the dominant pathogenic role. On the contrary, Allaqaband et al. (2002) reported that in patients with chronic renal insufficiency *N*-acetylcysteine or fenoldopam offered no additional benefit over hydration with saline in preventing radiocontrast-induced nephropathy.

Tepel et al. (2000) reported that *N*-acetylcysteine given prior to and the day of radiocontrast medium administration significantly reduced the risk of acute renal failure. Given the lack of other specific agents for preventing radiocontrast nephropathy (except NaCl loading), this positive finding has led to *N*-acetylcysteine's frequent use in the clinical setting. Because *N*-acetylcysteine can function as an antioxidant, it has been assumed that this action is responsible for its protective influence. However, *N*-

acetylcysteine may also attenuate radiocontrast media-induced renal vasoconstriction. Furthermore, it remains to be proven that *N*-acetylcysteine's protective action stems solely from free sulfhydryl versus its acetyl content (Safirstein et al. 2000). Given these considerations, *N*-acetylcysteine-mediated protection cannot necessarily be equated with an antioxidant effect expressed at the tubular cell level (Tepel et al. 2000).

It has been hypothesized that antioxidant compounds namely N-acetyl cysteine, ascorbic acid, glutathione and probucol may be effective due to their antioxidant properties (Lee et al. 2008). Results of Romano et al. (2008) supported the clinical observation of the effectiveness of *N*-acetylcysteine and ascorbic acid in preventing contrast-induced apoptosis. This effect is dose-dependent: indeed, the greater the dose, the larger the cellular benefit. This finding supports the clinical observation of the dose dependence of *N*-acetylcysteine in preventing contrast-induced nephropathy (Briguori et al. 2004). Of note, *N*-acetylcysteine was more effective against contrast-induced apoptosis than ascorbic acid. In contrast, sodium bicarbonate does not prevent contrast-induced apoptosis. However, clinical studies suggest that sodium bicarbonate seems to be effective at preventing contrast-induced nephropathy (Merten et al. 2004). Merten et al. (2004) reported that the combined prophylactic of sodium bicarbonate plus *N*-acetylcysteine, but not the combination of ascorbic acid and *N*-acetylcysteine, is more effective than *N*-acetylcysteine alone at preventing contrast-induced nephropathy. They speculated that *N*-acetylcysteine and ascorbic acid may work through similar pathways, while the protective action of bicarbonate may be different in comparison to *N*-acetylcysteine and, therefore, additive (Briguori et al. 2007). The lack of benefit of the combination of *N*-acetylcysteine and ascorbic acid in preventing contrast-induced apoptosis observed in the present study supports the hypothesis that free-radical formation is promoted by an acidic environment typical of distal tubular urine but inhibited by the higher pH of normal extracellular fluid (Boron 2006; Romano et al. 2008).

Erdosteine

Erdosteine, which is a thiol derivative, has been developed as a novel mucoactive agent that has antioxidant and anti-inflammatory properties and as an enhancer of respiratory ventilation in the treatment of patients with chronic obstructive respiratory disease (Demiralay et al. 2006). Several studies have also demonstrated that erdosteine is able to scavenge ROS (Ege et al. 2004). Hence, Yesildağ et al. (2009) investigated the protective role of erdosteine on radiocontrast media-induced nephrotoxicity in rats by evaluating antioxidant/oxidant and blood biochemical values. They observed dose-dependent protective effects of erdosteine on radiocontrast media-induced oxidative stress in the rat kidney. Similarly, Yesilyurt et al. (2011) investigated the protective role of erdosteine on radiocontrast media-induced kidney toxicity through total antioxidant capacity, total oxidant capacity and DNA damage in the rat kidney and observed significant protective effects.

Ascorbic Acid (Vitamin C)

Ascorbic acid is a low-molecular weight antioxidant well known as an antiscorbutic agent in humans, primates and guinea pigs. Spargias et al. (2004) reported that ascorbic acid, a safe, well-tolerated, inexpensive and readily available oral antioxidant, appears to prevent the complication of contrast-mediated nephropathy after invasive coronary imaging procedures in patients with preexisting renal dysfunction. Cetin et al. (2008) reported that ionic, high-osmolar contrast medium administration, either alone or together with antecedent cisplatin treatment, leads to accelerated oxidative reactions in rat kidney tissues against this oxidant stress.

Glutathione

Previous literature has supported the role of glutathione as a cytoprotective agent, but the mechanism is not yet understood. Previous studies have shown that exogenous glutathione treatment of renal cortical slices increased cellular glutathione concentrations (Harmon et al. 2005). This increase is likely mediated by uptake from the glutathione transporter located on the basolateral membrane of proximal tubular epithelial cells (Lash et al. 2005). Harmon et al. (2009) reported that glutathione reduced the toxicity of diatrizoic acid and iothalamic acid in renal cortical slices.

Conclusions

The present review provides the following new insights: radiocontrast media-induced cytotoxicity can be dissociated from tubular cell oxidant stress and Ca^{2+} influx. This is consistent with recent findings that antioxidants such as *N*-acetylcysteine, ascorbic acid and erdosteine may prevent clinical radiocontrast media-induced acute renal failure. The molecular basis for radiocontrast media-mediated cytotoxicity remains elusive at this time. However, release of inorganic iodide, with subsequent iodide toxicity appears not to be involved; and radiocontrast media can destabilize the plasma and potentially the mitochondrial membranes. This can result in loss of critical plasma membrane and mitochondrial (cytochrome *c*) proteins and an increase in plasma membrane susceptibility to Ca^{2+}

influx, phospholipase A_2 and ROS attacks. Contrast media induced overproduction of free oxygen radicals through activation of phagocytic cells. The formation of ROS also results from the evolving hypoxia and reoxygenation, activating a feed-forward loop of endothelial/vascular dysfunction, upregulation of tubular transport and induction of oxygen-consuming reparative mechanisms, with consequently intensified hypoxia. Via interference with hypoxia-adaptive cell responses, ROS might further intensify renal parenchymal injury and dysfunction. Given the importance of each of the above processes to cellular integrity, the current findings provide new potential insight into the mechanisms of radiocontrast media tubular toxicity and its associated acute renal failure. Whether the above observations, gathered using tubular cells, also have relevance to endothelial cells remains unknown at this time (Zager et al. 2003).

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