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*Minireviews*

**Contrast Induced Acute Kidney Injury and Direct Cytotoxicity of Iodinated Radiocontrast Media on Renal Proximal Tubule Cells**

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## 2. Running Title Page:

### a) Contrast Agent Cytotoxicity to the Proximal Tubule

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### d) Abbreviations:

ACEI, angiotensin-converting enzyme inhibitors; Akt, protein kinase B; ARB, angiotensin-II receptor blockers; CAT, catalase; CHF, congestive heart failure; CI-AKI, contrast-induced acute kidney injury; EGCG, epigallocatechin gallate; eGFR, estimated glomerular filtration rate; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; GFR, glomerular filtration rate; GPx, glutathione peroxidase; GSH, glutathione; HOcm, high-osmolar contrast media; IOCM, iso-osmolar contrast media; JAK, Janus kinase; JNK, c-Jun N-terminal kinase; LOCM, low-osmolar contrast media; MAPK, mitogen-activated protein kinase; MnSOD, manganese superoxide dismutase; NAC, N-acetyl cysteine; NO, nitric oxide; NSAID, nonsteroidal anti-inflammatory drug; Pink1, PTEN-induced putative kinase; PT, proximal tubule; RAAS, renin-angiotensin-aldosterone system; RCM, radiocontrast media; ROCK, Rho-associated protein kinase; ROS, reactive oxygen species; STAT, signal transducer and activator of transcription proteins; SCr, serum creatinine; TAL, thick ascending limb; UPR, unfolded protein response

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### 3. Abstract

The administration of intravenous iodinated radiocontrast media (RCM) to visualize internal structures during diagnostic procedures has increased exponentially since their first use in 1928. A serious side effect of RCM exposure is contrast-induced acute kidney injury (CI-AKI), which is defined as an abrupt and prolonged decline in renal function occurring 48 to 72 hours after injection. Multiple attempts have been made to decrease the toxicity of RCM by altering ionic strength and osmolarity, yet there is little evidence to substantiate that a specific RCM is superior in avoiding CI-AKI. RCM-associated kidney dysfunction is largely attributed to alterations in renal hemodynamics, specifically renal vasoconstriction; however, numerous studies indicate direct cytotoxicity as a source of epithelial damage. Exposure of *in vitro* renal proximal tubule cells to RCM has been shown to affect proximal tubule epithelium in the following manner: 1) changes to cellular morphology in the form of vacuolization; 2) increased production of reactive oxygen species (ROS) resulting in oxidative stress; 3) mitochondrial dysfunction resulting in decreased efficiency of the electron transport chain and ATP production; 4) perturbation of the protein folding capacity of the endoplasmic reticulum (ER) (activating the unfolded protein response (UPR) and inducing ER stress); 5) decreased activity of cell survival kinases. The present review focuses on the direct cytotoxicity of RCM on proximal tubule cells in the absence of *in vivo* complications such as alterations in renal hemodynamics or cytokine influence.

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#### 4. Introduction

Intravenous radiocontrast media (RCM) administration during procedures such as percutaneous coronary intervention and cardiac angiography is associated with contrast-induced acute kidney injury (CI-AKI). CI-AKI dysfunction ranges from asymptomatic increases in serum creatinine (SCr) to permanent renal damage requiring dialysis (Brown et al., 2016). CI-AKI is defined as a 0.5 mg/dl increase in SCr or a greater than 25% increase of baseline SCr peaking 3-5 days after administration and returning to baseline within 10-14 days in the absence of other contributing factors (Thomsen and Morcos, 2003; Mehran and Nikolsky, 2006). Although the exact mechanisms of RCM nephrotoxicity remains to be elucidated; potential mechanisms include altered renal hemodynamics and direct cytotoxicity to renal epithelium resulting in increased reactive oxygen species (ROS) and oxidative damage. Renal ischemia is a complex result of severe renal vasoconstriction, decreased oxygen supply, and increased oxygen demand within the renal outer medulla (Yang et al., 2018). Although a vast number of clinical reports and observational studies have explored the incidence of CI-AKI following administration of RCM, most finding significant risk, little is known about the direct cellular toxicity induced by RCM. To determine the source of RCM induced cytotoxicity, *in vitro* models must be implemented to eliminate renal hemodynamic and inflammatory responses to RCM administration. This minireview briefly discusses the characterization of RCM, renal pathogenesis, and risk factors involved in CI-AKI, as well as, the direct toxic effects of RCM on cellular antioxidant systems, mitochondria, the endoplasmic reticulum (ER) and the unfolded protein response (UPR), and activity of stress kinases in *in vitro* systems and briefly addresses current and experimental preventative measures for CI-AKI.

#### 5. Radiocontrast Media

The first RCM developed for intravenous administration: uroselectan, diodrast and hippuran were synthesized in the late 1920s for use in urography procedures (Swick, 1930). Further advances exploring the relationships between chemical structure, toxicity, and pharmacokinetics resulted in the discovery acetrizoic acid, the parent compound to all modern RCM agents (Wallingford, 1953). Continuation of these developments led to the detection of RCM that are much more tolerable and increasingly radiopaque compared to early radiopaque compounds.

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The first generation of modern RCM were ionic monomer derivatives of acetrizoic acid with osmolalities 5-8 fold greater than plasma, and denoted as high-osmolar contrast media (HOCM). The ionization of early RCM have been associated with many toxic reactions including: severe nephrotoxicity (Stokes and Bernard, 1961; Schreiner, 1966), induction of convulsions and seizures (Melartin et al., 1970), pancreatitis in patients undergoing endoscopic retrograde cholangio-pancreatography (Banerjee et al., 1990), severe and potentially fatal thrombi in patients with sickle-cell anemia (McNair, 1972), inhibition of platelet aggregation *in vivo* and *in vitro* (Gafer et al., 1979), and blood volume expansion leading to right heart volume overload in patients with poor cardiac function (Widmark, 2007). The theory that first generation RCM ionicity and osmolality were critical contributors to toxicity led to the dimerization or replacement of carboxyl groups with non-dissociating hydrophilic groups (Stratta et al., 2012). The resulting generation of RCM consisted of ionic dimers and non-ionic monomers that had osmolalities 2-3 fold greater than plasma and were labeled low-osmolar contrast media (LOCM). The practice of decreasing RCM osmolality continued resulting in the third generation of RCM of non-ionic-dimers that exhibit osmolality that is equivalent to plasma and are designated iso-osmolar contrast media (IOCM).

Following intravenous administration, RCM are rapidly diluted and distributed by circulating plasma reaching concentrations adequate for visualization in approximately 5-10 minutes. First generation RCM have low serum-albumin binding of 0-10% while second and third generation RCM show no notable serum protein binding. RCM of all generations show no significant metabolism and are excreted unchanged predominately through the kidneys by glomerular filtration with only 2% recovered in the feces (GE-Healthcare, 2006; Amersham-Health, 2007). The pharmacokinetics of RCM can be described by a two-compartment model consisting of a rapid distribution (alpha) phase and a slow elimination (beta) phase. For example, in patients with normal kidney function, the alpha and beta phase half-lives for diatrizoic acid are 30 minutes and 120 minutes, respectively. However, in patients with significant renal impairment, the beta phase half-life can be prolonged for several days (Amersham-Health, 2007).

The indications for RCM change in accordance to procedure and risk of the patient involved. All generations of RCM are used for angiocardiology, although the use of HOCM is not

recommended in patients with renal or cardiac insufficiencies. HOCM are used specifically for nonvascular procedures such as pyelography, cystography, and routine computed tomography procedures. LOCM and IOCM are used for vascular procedures and multi-detector computed tomography (American College of Radiology Committee on Drugs and Contrast Media, 2017). It is perceived that a major portion of the toxicity of RCM is due to elevated osmolalities, however, reducing the osmolality of RCM results in a substantial increase in RCM viscosity (Figure 1). High injection rates of RCM with higher viscosities could be a contributing factor in stasis of renal tubular function (Ueda et al., 1993). Although there is considerable dissension as to which generation of RCM is the safest, IOCM are thought to be optimal as they deliver the most iodine per molecule with the least impact on osmolality. Figure 2 describes the physical structures of various RCM. Table 1 summarizes several parameters of the various generations of RCM.

## 6. Renal Pathogenesis

The administration of intravenous iodinated RCM for diagnostic procedures and medical interventions have increased exponentially since their introduction in the late 1920s. Unfortunately, CI-AKI is the third most common cause of hospital-acquired kidney damage, accounting for 12% of cases (Nash et al., 2002; Gleeson and Bulugahapitiya, 2004). The level of this dysfunction can vary from a mild increase in SCr to permanent renal failure resulting in dialysis (Brown et al., 2016). Although the definition of CI-AKI varies in the literature, it is generally defined as an absolute increase in SCr greater than 0.5 mg/dl or a relative increase of greater than 25% of baseline SCr peaking 3-5 days after administration and returning to baseline within 10-14 days (Thomsen and Morcos, 2003; Mehran and Nikolsky, 2006). The incidence of CI-AKI is low, less than 2%, in patients with normal to slightly diminished renal function ( $GFR > 60 \text{ ml/min per } 1.73 \text{ m}^2$ ) (Berns, 1989; Weisbord and Palevsky, 2008); however, in high-risk patients with moderate to severe renal impairment the incidence is as high as 55% (Mehran and Nikolsky, 2006).

Although the exact mechanisms of toxicity of RCM have not been fully elucidated, it is probable that the acute deterioration of kidney function is caused by a combination of renal medullary hypoxia and direct toxicity to renal epithelial cells. Intravenous administration of RCM induces transient vasodilation of renal vasculature followed by severe, sustained vasoconstriction,

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specifically of the afferent arterioles, resulting in decreased oxygen supply and oxidative damage to the outer medulla (Persson et al., 2005; Liu et al., 2012). This response is likely due to modifications of endogenous renal vasomodulators, specifically nitric oxide (NO), endothelin, and adenosine (Hall et al., 1992). The production of the vasodilator NO decreases in response to RCM resulting in exacerbation of renal vasoconstriction (Touati et al., 1993). Levels of endothelin, a vasoconstrictive peptide released by endothelial cells to maintain blood pressure, have been shown to increase after RCM exposure (Clark et al., 1997). It is also apparent that adenosine plays a role in inducing CI-AKI. Intrarenally administered adenosine produces biphasic hemodynamic alterations that are similar to those induced by RCM (Arend et al., 1987).

The oxidative damage induced by vasoconstriction is compounded in the relatively hypoxic renal medullae. Under normal physiological function, the medulla functions at very low O<sub>2</sub> tension as the result of limited blood flow and the ‘oxygen shunt’ caused by the anatomy of the outer medullary vasculature (Heyman et al., 1997; Leong et al., 2007). Along with poor O<sub>2</sub> delivery, O<sub>2</sub> consumption within the outer medullae is high due to ion reabsorption by the thick ascending limb (TAL) of the nephron (Heyman et al., 1997). The combined effects of low O<sub>2</sub> delivery and high O<sub>2</sub> consumption renders the renal medullae particularly susceptible to hypoxic injury. RCM exposure has been shown to further decrease O<sub>2</sub> tension within the renal medulla and simultaneously induce osmotic diuresis and increase ion transport and O<sub>2</sub> consumption at the TAL (Heyman et al., 1991; Cronin, 2010). Additionally, an increase in tubular pressure secondary to RCM induced diuresis, increased urinary viscosity, and tubular obstruction result in increased oxygen consumption, worsening ischemic damage (Gleeson and Bulugahapitiya, 2004). The combination of alterations in vasomodulators and increased oxidative damage may exert direct tubular and vascular endothelial damage leading to a vicious cycle of hypoxic damage in the renal parenchyma (Heyman et al., 2010). Although renal papillary necrosis is not a hallmark of CI-AKI, administration of RCM is contraindicated in patients that are undergoing selective or nonselective nonsteroidal anti-inflammatory drug (NSAID) therapy. Concomitant use may aggravate NSAID induced renal vasoconstriction, therefore, NSAID therapy should be suspended 48 hours prior to RCM administration (Horl, 2010).

## 7. Risk Factors

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Individuals with chronic kidney disease associated with diabetes have the greatest risk for CI-AKI. Other factors which increased the risk for CI-AKI (Table 2) include congestive heart failure (CHF), salt depletion/dehydration, prolonged hypotension, low hematocrit, age > 70 years, multiple myeloma, sepsis, and concurrent use of certain drugs. Advanced age is an independent predictor of CI-AKI (Gussenhoven et al., 1991). Age-related alterations in renal function including decreases in GFR, tubular secretion, and urine concentrating ability (Sands, 2012; Denic et al., 2016) play a role, as well as the requirement for larger doses of RCM in elderly patients with calcified vasculature (Mehran and Nikolsky, 2006).

Preexisting renal dysfunction is a serious risk factor for CI-AKI development. Due to the inverse relationship of estimated glomerular filtration rate (eGFR) and the risk for CI-AKI, the defined eGFR cut-off point for classifying patients as high risk is  $\leq 60$  mL/min/1.73 m<sup>2</sup> (Tsai et al., 2014). The incidence of CI-AKI in patients with underlying chronic renal failure ranges from 14.8 to 55%, and risk can double in patients with concomitant diabetes mellitus when compared to nondiabetic patients (Mehran and Nikolsky, 2006).

Any extrarenal condition that effectively lowers intravascular volume profoundly increases the risk of CI-AKI. Conditions that reduce effective intravascular volume include: CHF, prolonged dehydration, and salt depletion. CHF activates numerous humoral and neurohumoral mechanisms resulting in sodium and water reabsorption by the kidneys. These mechanisms result in expansion of extracellular fluid by increasing venous capillary pressure, decreasing plasma oncotic pressure, and promoting fluid extravasation and edema formation (Navas and Martinez-Maldonado, 1993). Compromised left ventricle systolic performance often apparent in CHF patients has also been linked to increased risk of CI-AKI (Martin-Paredero et al., 1983; Gruberg et al., 2000). Severe dehydration and salt depletion secondary to abnormal fluid losses associated with insufficient salt intake have been shown to play a role in CI-AKI (Detrenis et al., 2005). Prolonged hypotension as a result of decreased effective intravascular volume or induced by antihypertensive treatment using angiotensin-converting enzyme inhibitors (ACEI), angiotensin-II receptor blockers (ARB), or diuretics heighten risk for CI-AKI (Barrett and Parfrey, 1994). Decreased effective intravascular volume contributes to CI-AKI risk by reducing renal perfusion

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and GFR resulting in decreased O<sub>2</sub> delivery and increasing the concentration of RCM within the renal tubules furthering ischemic insult and direct cytotoxicity.

Anemia, or low hematocrit, is an independent risk factor for CI-AKI (Nikolsky et al., 2005; Cho et al., 2010; Murakami et al., 2013). A study of 510 patients indicated that individuals with lower hematocrit were more likely to develop CI-AKI (Cho et al., 2010). In an earlier study with patients who displayed both reduced eGFR and hematocrit, rates of CI-AKI were as high as 28.8%, whereas patients with only a diminished eGFR and normal hematocrit had significantly lower rates of CI-AKI (Nikolsky et al., 2005). The mechanism for increased CI-AKI risk in patients with low hematocrit may be partially attributed to diminished oxygen delivery to the kidney which could increase susceptibility to renal damage. A study of 200 patients revealed less CI-AKI in patients provided oxygen 10 minutes prior to angiography compared to room air (Sekiguchi et al., 2018). These findings suggest sufficient oxygenation may be beneficial to reduce renal damage by RCM, but further studies are warranted to explore the beneficial effect of oxygen.

Many radiologists and physicians consider RCM use a contraindication in patients with multiple myeloma (Bartels et al., 1954; Killmann et al., 1957; Scheitlin et al., 1960). CI-AKI in myeloma patients has been attributed to precipitation of RCM with Tamm-Horsfall glycoprotein, resulting in increased ischemic injury and desquamation of renal proximal tubule (PT) cells (Dawnay et al., 1985). A more recent retrospective study reviewed 46 patients with myeloma who underwent RCM-enhanced CT scans of the chest, abdomen, and pelvis (Pahade et al., 2011). Of the 46 patients, 12 (26.1%) developed CI-AKI; however, there was no significant difference in peak SCr levels in patients that obtained CI-AKI when compared to the patients that did not. Of the parameters obtained, only serum levels of  $\beta$ 2-microglobulin increase in both patients with high tumor burden and decreased renal function indicating a statistically significant correlation with the development of CI-AKI. According to the authors, a threshold value of <2.8mg/L of  $\beta$ 2-microglobulin could essentially eliminate the risk of CI-AKI in patients with multiple myeloma. The European Society of Urogenital Radiology (ESUR) concluded multiple myeloma was not a risk factor following evaluation of a retrospective study of 13 studies (Stacul et al., 2018). However, ESUR concluded that myeloma patients with diminished renal function, dehydration, or hypercalcemia were at a much higher risk for CI-AKI (Stacul et al., 2018).

Sepsis is a life-threatening medical condition most often caused by an overwhelming immune response to a body-wide bacterial infection. Sepsis affects both adult and pediatric patients and is associated with numerous changes to renal function that may be mediated by release of these inflammatory agents as well as direct tubular insult (Alobaidi et al., 2015). Pro-inflammatory cytokines are released into the bloodstream to combat the infection triggering widespread inflammation, thrombus formation, and endothelial damage (Cavaillon et al., 2003). In cases of septic shock, side-effects include: reduced blood pressure, abnormalities in microcirculatory blood flow, coagulopathy, and endothelial dysfunction resulting in severely reduced oxygen delivery and organ dysfunction or failure (Angus and van der Poll, 2013). However, further studies are needed to evaluate the contribution of renal perfusion as animal studies and human studies have reported both increased and decreased renal blood flow (Langenberg et al., 2005; Langenberg et al., 2006). Autopsy series fail to detect extensive cellular necrosis in victims of septic shock (Takasu et al., 2013); this may be due to sepsis promoting mitochondrial damage in renal tubular epithelial cells resulting in decrease oxygen consumption (Tran et al., 2011). In addition to decreased oxygenation, renal epithelium experience activation of an innate immune response, giving rise to pathogenic oxidative stress and further tissue injury (Wiersinga et al., 2014). The use of nephrotoxic antibiotics to combat the infection can complicate the diagnosis and treatment of sepsis-associated acute kidney injury. Perhaps one, or all, of these factors play a role in why patients undergoing radiopaque imaging procedures are at increased risk of sepsis (Matejovic et al., 2011). Due to the bidirectional nature of sepsis, additional studies are needed to evaluate the connections between CI-AKI and sepsis.

Concomitant use of nephrotoxic drugs increases the risk of CI-AKI. Aminoglycosides, cyclosporine A, amphotericin B, and cisplatin increased kidney susceptibility to RCM-induced toxicity (Kolonko et al., 1998; Morcos, 1998). Aminoglycosides and amphotericin B exert a direct nephrotoxic effect via disruption of normal phospholipid trafficking and the induction of severe renal vasoconstriction (Swan, 1997; Fanos and Cataldi, 2000). Cyclosporin A impairs lysosomal function and damages both proximal and distal convoluted tubules resulting in changes to tubulointerstitial transport (Kolonko et al., 1998). Cisplatin, and other platinum derivatives, impair enzyme function by binding to sulfhydryl groups resulting in PT cytotoxicity (Hanigan and

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Devarajan, 2003). Long-term ingestion of high doses of NSAIDs, certain penicillins, and sulfonamides can lead to acute tubulointerstitial nephritis which increases the risk of CI-AKI (Kolonko et al., 1998; Morcos, 1998). Although the role of ACEIs and ARBs in CI-AKI is controversial, many providers avoid concomitant use (Toprak, 2007). According to the Council on the Kidney in Cardiovascular Disease and the Council for High Blood Pressure Research of the American Heart Association, the use of ACEIs or ARBs in radiopaque imaging procedures depends on multiple factors. ACEIs or ARBs are acceptable and can even improve renal hemodynamics and salt excretion provided the patient's renal perfusion pressure is adequate and volume depletion is minimal. However, angiotensin II is essential in the autoregulation of GFR and renal perfusion, and the use of these agents in hypovolemic patients can induce a dramatic decrease in GFR and subsequent oliguric or anuric renal failure (Schoolwerth et al., 2001). Conversely, the Kidney Disease Improving Global Outcomes Clinical Practice Guideline for Acute Kidney Injury states that there is not enough evidence to recommend the discontinuation of ACEIs or ARBs prior to RCM administration (KDIGO, 2012). Loop diuretics, specifically furosemide, have been shown to increase the risk of CI-AKI (Majumdar et al., 2009). Other studies have stated that although loop diuretic exposure is associated with acute kidney injury in hospitalized patients, the change in renal function is small and loop diuretic use does not explain the variability in overall renal function (El-Refai et al., 2011).

## **8. Cytotoxicity**

The cytotoxic effects of RCM on PT cells have been characterized by vacuoles in the PT cells, increased production of ROS and induction of oxidative stress, mitochondrial dysfunction and an ensuing decline in ATP production, activation of the UPR and ER stress, and abnormal stress kinase activities (Tervahartiala et al., 1991; Andersen et al., 1994; Andersen et al., 1995; Tervahartiala et al., 1997). Epithelial PT cell vacuolization is an indicator of drug toxicity and a histopathological feature of CI-AKI. One route in which RCM are influxed into PT cells is via pinocytosis. During the process of urine production, RCM is concentrated in the renal tubules. As a result, PT cells are exposed to increasing concentrations of RCM, inducing a rise in pinocytic vesicles containing RCM, formation of large vacuoles, alterations in renal histology, and epithelial damage (Dickenmann et al., 2008; Andreucci et al., 2014). An early study on vacuolization of PT in response to RCM demonstrated that male Sabra rats experienced vacuolar changes in the early

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segments of the PT within 120 minutes of administration to 2.9 g I/kg of iohalamate. The changes were described as being similar in nature and closely resembled what has been described in human contrast nephropathy (Heyman et al., 1988). A study performed by Tervahartiala et al. demonstrated that 3 g I/kg of diatrizoate or iopromide induced statistically significant PT cell vacuolization within 2 hours of administration in diabetic Wistar rats of both sexes (Tervahartiala et al., 1991). Iohexol and iotrolan caused longer lasting and more pronounced vacuolization (Tervahartiala et al., 1991). A separate study on male Sprague-Dawley rats showed a low-level increase in cytoplasmic vacuoles in PT cells occurred in as little as 5 minutes and continued to increase in size and number for 24 hours after single-dose administration of 3 g I/kg iotrolan (Rees et al., 1997). A more modern study performed on male Wistar rats demonstrated that a concentration as low as 10ml/kg of iodixanol can induce vacuolization in PT cells in 24 hours (Nasri et al., 2015). Although RCM from each generation induce vacuolization, the lack of *in vitro* studies makes it impossible to determine if this is a direct toxic side effect of RCM.

CI-AKI is the result of a combination of alterations in renal hemodynamics and toxic renal parenchymal damage. Aside from hypoxia induced by low oxygen supply, it is believed that the latter of the two is due to oxidative stress induced by an increase in ROS. Additionally, exposure to RCM results in diminished availability and activity of cellular antioxidant systems. Under physiological conditions, the production of ROS within renal parenchyma is connected to tubular transport, specifically in areas of the nephron that have dense mitochondrial populations such as the PT, the convoluted tubules, or the TAL of the nephron. A major source of production of superoxide anions ( $O_2^{\bullet-}$ ) and hydroxyl radicals ( $OH^{\bullet}$ ) takes place within the mitochondria via NAD(P)H-oxidases (Heyman et al., 2010). Additionally, as the consumption of oxygen begins to overwhelm available oxygen during periods of hypoxia, there is an increase in the complete conversion of ATP to hypoxanthine by 5'-nucleotidase. From this, hypoxanthine is converted to uric acid and hydrogen peroxide ( $H_2O_2$ ) via xanthine oxidase. Consequently,  $H_2O_2$  and  $O_2^{\bullet-}$  can react with NO to form peroxynitrite leading to renal vasoconstriction and epithelial damage by decreasing the vasodilatory effects of NO and interacting with protein and DNA, respectively. Although there is evidence that peroxynitrite can induce a degree of vasodilation initially, long-term exposure to peroxynitrite in an environment devoid of NO can lead to irreversible endothelial and epithelial damage (Pacher et al., 2007).

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Several experimental studies have shown that RCM enhances renal ROS production resulting in oxidative stress. Huang and colleagues noted that exposure of a non-cancerous, immortalized human renal proximal tubule epithelial cell line (HK-2) to the HOCM ioxitalamate induced an increase in cytoplasmic ROS production and formation of 8-hydroxy-2'-deoxyguanosine (8-OHdG), indicating oxidative damage following a 48 hour treatment (Huang et al., 2016). A significant increase of cytosolic superoxide formation took place only 2 hours after exposure of HK-2 cells to 150 mg I/mL of the LOCM iohexol (Jeong et al., 2018). A similar study using a canine distal convoluted tubule cell line (MDCK) showed that exposure to 50, 100, or 200 mg I/mL of iobitridol, iopamidol, or iodixanol induced a dose-dependent increase in ROS production within 3 hours (Quintavalle et al., 2011). A study performed by Netti et al. demonstrated that a statistically significant increase NADPH oxidase-dependent ROS can be seen in HK-2 cells within 30 minutes of exposure to 200 mg I/mL of LOCM iohexol or iopamidol (Netti et al., 2014). An increase in intracellular ROS can be seen regardless of generation of RCM.

Although there are several *in vitro* studies pertaining to the effect of RCM on the production of ROS, very little has been done to examine the source of ROS production. For instance, a rise in ROS concentrations could be, in part, due to a reduction in the efficacy of intracellular antioxidant systems. Figure 3 shows the intracellular antioxidant systems. At the time of this review, a study performed by Jeong et al. is a lone study examining RCM effects on intracellular antioxidant systems *in vitro*. The Jeong group determined that HK-2 cells exposed to 150 mg I/mL of iohexol induced a significant decrease in manganese superoxide dismutase (MnSOD) and glutathione peroxidase (GPx) after 2 hours (Jeong et al., 2018). It is apparent that there is a significant gap in knowledge pertaining to the effects of RCM on antioxidant systems *in vitro*; however, the effects of RCM on reduced glutathione (GSH), GPx, MnSOD, and Catalase (CAT), have been studied extensively *in vivo*. A study performed by Gong et al. determined that male Sprague-Dawley rats exposed to 1.5-2 g I/kg of iohexol resulted in a decrease MnSOD and GSH after 24 hours (Gong et al., 2016). Tasanarong et al. showed in a similar study that exposing male Sprague-Dawley rats to 1.6 g I/kg of iopromide induced a significant decrease in MnSOD and CAT after 24 hours (Tasanarong et al., 2014). GSH, GPx, MnSOD, and CAT were all significantly reduced in male Sprague-Dawley rats exposed to 3 g I/kg of iodixanol (Liu et al., 2018). It should be stated that these changes in cellular oxidant defenses may not be a direct result of RCM toxicity and could be

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induced, in part, by alterations in renal hemodynamics. Taken together, increased ROS production and a decrease in the efficacy of renal antioxidant systems plays an important role in CI-AKI; however, the effects of RCM on antioxidant systems on in vitro systems needs to be studied more in depth.

Under normal physiological conditions, the mitochondria are one of the main sources of intracellular ROS and the main organelle target for ROS (Cho et al., 2010). Previous studies indicate that mitochondrial injury plays an important role in various types of acute kidney injury (Xiao et al., 2014; Tang et al., 2018). Humes et al. noted that PT segments isolated from New Zealand white rabbits incubated with diatrizoate demonstrated significant reductions in basal and uncoupled respiration and intracellular ATP levels indicating an interaction between diatrizoate and PT cell mitochondria (Humes et al., 1987). A follow up study using the same model compared the mitochondrial toxicity of diatrizoate and iopamidol (Messana et al., 1988). The authors determined that diatrizoate induced greater reductions in intracellular ATP, basal respiration, and uncoupled respiration when compared to iopamidol and both RCM were toxic when compared to control. Messana et al. continued their studies of the effects of RCM on isolated proximal tubules by comparing the mitochondrial toxicity of the non-ionic monomer iopamidol and ionic dimer ioxaglate. The authors determined that both RCM induced mitochondrial damage but the differences in direct nephrotoxic potential become insignificant when normalized for iodine content (Messana et al., 1990). Exposure of a porcine kidney cell line that express characteristics similar to PT cells (LLC-PK1) to ioversol caused depolarization of mitochondrial membranes and stimulated the release of cytochrome c activating caspase-9 through the action of the adaptor molecule apoptotic protease-activating factor-1 (Itoh et al., 2006). Lei et al. determined that exposure to iohexol or iodixanol induced a significant increase in mitochondrial ROS and mitochondrial membrane potential in HK-2 cells (Lei et al., 2018). An *in vivo* investigation on the effects of RCM on the electron transport chain determined that exposure of male Wistar albino rats to 1.95 g I/mL of diatrizoate tended to decrease renal complex I and complex III activity, although not to a significant degree (Roza et al., 2011). As one would expect, significant mitochondrial dysfunction results insufficient ATP for a cell to maintain its cellular functions resulting in activation of the intrinsic apoptotic pathways; however, the source of mitochondrial dysfunction in response to RCM exposure has yet to be identified (Haller and Hizoh, 2004).

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The primary role of the ER is the synthesis and folding of secreted, membrane-bound, and some organelle-targeted proteins. The environment within the ER is optimal for protein folding and management, as well as ATP storage and calcium transport (Gaut and Hendershot, 1993). Perturbations of cellular ATP levels, calcium concentration, and/or the redox status within a cell can lead to a decline in the protein folding ability of the ER (Bravo et al., 2013). An accumulation of unfolded or misfolded proteins activates a pro-survival response responsible for restoring protein folding function within the ER called the unfolded protein response (UPR) (Schroder and Kaufman, 2005). Prolonged activation of the UPR will promote a shift from pro-survival to pro-apoptotic signaling, designated ER stress (Szegezdi et al., 2006). ER stress can lead to the activation of multiple signaling pathways that induce cellular apoptosis (Figure 4) and its role in CI-AKI has recently become a topic of interest. An initial study performed by Wu et al. determined the effects of diatrizoate on rat renal proximal tubule cells (NRK52E). Exposure of NRK52E cells to 40 mg I/mL of diatrizoate induced an increase in multiple ER stress markers including glucose related peptide-78 (GRP78), protein kinase RNA-like ER kinase (PERK), and inositol requiring protein-1 (IRE1) within 24 hours (Wu et al., 2010). An additional study by Wu et al. on NRK52E cells determined that 40 mg I/mL of diatrizoate induced significant increases in the ER stress markers activating transcription factor 6 (ATF6), C/EBP homologous protein (CHOP), and the ER stress dependent caspase, caspase-12 within 24 hours (Wu et al., 2013). Peng et al. showed that HK-2 cells exposed to 40 mg I/mL of diatrizoate induced statistically different increases in GRP78, activating transcription factor 4 (ATF4), CHOP, and caspase-12 within 4 hours (Peng et al., 2015).

Mitogen-activated protein kinases (MAPKs) such as the extracellular signal-regulated kinases (ERK1/2 or p42/44MAPK), the c-Jun N-terminal kinases (JNK1/2), and p38MAPK have been shown to be activated in response to ROS (Robinson and Cobb, 1997; Matsuzawa and Ichijo, 2008). The ERK1/2 cascade is activated in response to mitogenic and survival stimuli, whereas JNK1/2 and p38MAPK pathways are stimulated in response to cellular stresses including oxidative agents, hypoxia, UV radiation, and pro-inflammatory cytokines (Ip and Davis, 1998; Ichijo, 1999). Previous studies have shown that renal epithelial cell exposure to RCM results in activation of the intrinsic apoptotic pathway (Romano et al., 2008). An extensive study performed by Quintavalle et al. demonstrated the role of stress kinases in the activation of this pathway. MDCK cells were exposed to 50, 100, or 200 mg I/mL iobitridol, iopamidol, or iodixanol for 3 hours to determine

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the effect of RCM on stress kinases. Each of the tested RCM induced a dose-dependent increase in phosphorylated JNK and p38MAPK within 1 hour, indicating that exposure to RCM does induce activation of the pro-apoptotic stress kinases. The role of JNK and p38MAPK were verified in two methods: pretreating the MDCK cells with the p38 inhibitor SB203580 or transfecting MDCK cells with kinase-dead mutants for p38 or JNK. MDCK cells pretreated with SB203580 or transfected with kinase-dead mutants were completely protected from apoptotic effects of all three RCM verifying the role of stress kinases in CI-AKI (Quintavalle et al., 2011). Another study confirming Quintavalle's findings demonstrated that pretreating HK-2 cells with JNK inhibitor SP600125 or p38 inhibitor SP283580 significantly decreased cleaved caspase-3 activation and increased pro-survival protein bcl-2 after exposure to 200 mg I/mL iopamidol (He et al., 2016). A more recent study on LLC-PK1 cells determined that 25 mg I/mL of iodixanol will induce increases in activated p38MAPK, JNK, and ERK after 24 hours of exposure (Lee et al., 2018).

Additional kinases important to cellular survival that are affected by RCM exposure include protein kinase B (Akt), PTEN-induced putative kinase (Pink1), Rho-associated protein kinase (ROCK), Janus kinases (JAK) and signal transducer and activator of transcription proteins (STATs). A study performed by Xie et al. on the effects of ioversol on HK-2 cells indicated that a 30 minute exposure to 100 mg/ml of ioversol significantly decreases the presence of phosphorylated Akt while simultaneously inducing caspase-3 cleavage and apoptosis (Xie et al., 2017). This discovery demonstrates that the phosphoinositide-3 kinase (PI3)/Akt pathway may play a role in cellular fate after exposure to RCM. Renal tissues extracted from male Sprague-Dawley rats that were exposed to 12.25 g I/kg iohexol demonstrated a significant increase of Pink1 (Yang et al., 2018). Activation of Pink1 indicates an increase in mitophagy resulting in decreased ATP production and energy failure. ROCK plays an important role in various cellular processes including: cell adhesion, migration, proliferation, cytokine activation, inflammatory cell migration, smooth muscle cell contraction and cell cycle regulation. Inhibition of Rho-ROCK pathways has been shown to diminish CI-AKI *in vivo* (Su et al., 2014; Wang et al., 2018); however, the relationship of CI-AKI and ROCK has not been explored *in vitro*. The role of JAK-STAT pathway was determined in a study performed by Yokomaku et al. Pretreating male Sprague-Dawley rats or LLC-PK1 cells with asialoerythropoietin or erythropoietin activated JAK2 and

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STAT5 and attenuated ioversol-induced nephropathy or cellular injury indicating that the JAK-STAT pathway may play a role in CI-AKI (Yokomaku et al., 2008).

Calcium overload within renal parenchyma is also thought to play a role in CI-AKI via ROS overproduction, p38 MAPK activation, and endothelin activation and release (Humes et al., 1987; Duan et al., 2000; Schick et al., 2002; Yang and Yang, 2013; Jin et al., 2015). However, the initial source of intracellular calcium overload have not been fully elucidated and could result from ER or mitochondrial dysfunction, inhibition of the Na/K-ATPase, or changes in function of the Na/Ca exchanger system (Yang and Yang, 2013; Yang et al., 2013; Krebs et al., 2015).

### **9. Interventions for Cytotoxicity by Hydration, Drugs, and Natural Products**

Adequate hydration is the gold standard for prevention of CI-AKI. The rationale is that forced or adequate hydration induces an expansion of intravascular volume, suppression of renin-angiotensin cascade, reduction of renal vasoconstriction and hypoperfusion, and increases urine output (Andreucci et al., 2014). Higher urine output is associated with a lower incidence of CI-AKI due to the decreased time of tubular RCM exposure (Solomon and Dauerman, 2010). Unfortunately, high infusion rates can result in a volume overload and initiate pulmonary edema in patients with preexisting cardiac impairment (Chen et al., 2018). Substantial current research has focused on developing a potential nephroprotective agent.

The most explored strategy in the prevention of CI-AKI is the use of antioxidants to decrease contrast-induced oxidative stress. Common antioxidants have been studied including N-acetylcysteine (NAC), ascorbic acid, and  $\alpha$ - $\gamma$ -tocopherol. *In vitro* studies have provided consistent results for protection by NAC. NAC was protective for IOCM and LOCM cytotoxicity in LLC-PK1, MDCK, and human embryonic kidney (HEK-293) cells. At a concentration of 200 mg I/mL of iobitridol and iodixanol and an incubation time of 3 hours, pre-treatment with NAC protected the three cell lines from RCM-induced apoptosis (Romano et al., 2008). Yang et al. demonstrated that NRK-52E cells pretreated with low concentration NAC (10 mmol/L) before being exposed to 50, 100, and 150 mg I/mL of iopromide for 4 hours significantly decreased early stage apoptosis as seen by Annexin V and propidium iodide (PI) staining (Yang et al., 2014). The Romano et al. study mentioned above also examined the protective effects of ascorbic acid (vitamin C) *in vitro*.

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The authors demonstrated that a 2 hour pretreatment with ascorbic acid prevented high concentration IOCM- and LOCM-induced apoptosis in a dose-dependent fashion; however, NAC was more effective than ascorbic acid at preventing RCM-induced cytotoxicity (Romano et al., 2008). Unfortunately, the protective effects of  $\alpha$ -/ $\gamma$ -tocopherol on CI-AKI have not been studied *in vitro*.

Although certain antioxidants have shown to be protective against CI-AKI *in vitro*, the results from a clinical standpoint are inconclusive. Numerous clinical studies have shown NAC to be a potentially effective strategy for the prevention of CI-AKI (Tepel et al., 2000; Briguori et al., 2004; Briguori et al., 2007), other studies have concluded that NAC alone or in combination with ascorbic acid was not beneficial to patients (Yeganehkah et al., 2014; Palli et al., 2017). Further studies are needed to evaluate the long-term clinical benefits in patients as a study in over 5000 patients concluded that NAC was not beneficial when evaluating outcomes 90 days after angiography in high renal risk patients (Weisbord et al., 2018). Antioxidant vitamins  $\alpha$ -/ $\gamma$ -tocopherol (Vitamin E) have also been shown to be useful in the prevention of CI-AKI in a clinical setting (Spargias et al., 2004; Briguori et al., 2007; Tasanarong et al., 2013). A study performed by Kongkham et al. on male Sprague-Dawley rats demonstrated that pretreatment with 250 or 500 mg Vitamin E prior to induction of CI-AKI with 1.6 g I/kg iopromide significantly decreased tubular necrosis, PT cell congestion, and interstitial edema while simultaneously increasing total antioxidant capacity and MnSOD activity when compared to non-pretreated rats (Kongkham et al., 2013).

HMG-CoA reductase inhibitors, or statins, are a class of lipid lowering medications specifically used in the treatment of cardiovascular disease. Statins are thought to protect against RCM cytotoxicity by increasing heme oxygenase-1 production and diminishing the activity of NADPH oxidase, resulting in the reduction of ROS formation and oxidative stress induced by RCM (Grosser et al., 2004; Stoll et al., 2004). Cell culture results have been promising as MDCK and HK-2 cells pretreated with 0.2  $\mu$ M atorvastatin for at least 6 hours had higher cell viability and subsequent decrease in RCM-induced apoptosis after a 3 hour exposure to 200 mg I/mL iodixanol (Quintavalle et al., 2012). Initial clinical studies suggest statin pretreatment may reduce CI-AKI especially in high risk patients (Jo et al., 2008; Toso et al., 2010; Tropeano et al., 2016).

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Pharmacologic agents used primarily for the treatment of hypertension such as nebivolol, ACEIs, and ARBs have garnered some interest in the prevention of CI-AKI. Nebivolol is a third generation  $\beta_1$ -receptor antagonist that may protect the kidney via its antioxidant and NO-mediated vasodilating properties (Toprak et al., 2008). Toprak et al. determined the efficacy of nebivolol in preventing CI-AKI initially in female Wistar albino rats pretreated with 2 mg/kg of nebivolol once daily for 5 consecutive days prior to administration of 6 ml/kg of diatrizoate on the fourth day. Pretreatment with nebivolol resulted in a reduction of tubular necrosis, medullary congestion, and tubular casts, as well as an increase renal NO levels in rats when compared to rats that did not receive nebivolol (Toprak et al., 2008). A more recent study followed the Toprak et al. study design and determined that pretreatment with nebivolol prior to administration of RCM significantly reduced advanced oxidation protein products and malondialdehyde in serum and kidney tissue and increased total serum NO concentration (Koc et al., 2011). The role of the renin-angiotensin-aldosterone system (RAAS) and RAAS-blocking drugs in CI-AKI is controversial. This is due to experimental data suggesting that ACEIs or ARBs protect the kidneys from RCM-induced nephrotoxicity while other claims that RAAS-blocking drugs are nephrotoxic and worsen CI-AKI (Ikeda et al., 2006; Rosenstock et al., 2008; Patel et al., 2011). A large prospective study performed by Holscher et al. evaluated CI-AKI predictors and long-term outcomes of high-risk patients. The authors noted that decreased eGFR, the use of ACEIs, and post-procedural hemodialysis were independently associated with increased incidence of CI-AKI (Holscher et al., 2008). The overall lack of *in vitro* experimentation pertaining to high blood pressure medications is troubling when compared to the amount of clinical data that has been collected. This is an area where more research is necessary to make an accurate prediction of the possible mechanisms involved.

The administration of naturally derived compounds in the prevention of CI-AKI has shown considerable promise in recent years. *In vitro* polyphenolic compounds such as resveratrol, salvianolic acid B, epigallocatechin gallate (EGCG), flavonoids compound found in *Artemisia argyi*, the xanthone compound  $\alpha$ -mangostin all demonstrated protective effects against CI-AKI. Resveratrol, a compound found in grape skins, significantly reduced HK-2 cytotoxicity induced by ioxithalamate after 48 hour exposure (Huang et al., 2016). Salvianolic acid B, derived from the traditional Chinese medicine Danshen, decreased renal epithelial damage in male Sprague-Dawley rats exposed to iohexol (Tongqiang et al., 2016). In a similar fashion to salvianolic acid B, EGCG,

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the major antioxidant component of green tea, reduced the damage induced by iopromide in male Sprague-Dawley rats (Gao et al., 2016). A study performed by Lee et al. demonstrated that the flavonoid components of *Artemisia argyi*, also called mugwort, completely prevented the cytotoxic effects induced by iodixanol on LLC-PK1 cells (Lee et al., 2018). A separate study performed by Lee et al. showed that non-toxic concentrations of  $\alpha$ -mangostin, found in mangosteen, improved the viability of iodixanol treated LLC-PK1 cells by 90.42% against contrast-induced apoptotic damage (Lee et al., 2016). It is apparent that the nephroprotective effects of certain naturally derived compounds can prevent contrast-induced cytotoxicity both *in vitro* and *in vivo*.

## 10. Conclusions

RCM are non-biologically active compounds necessary for a multitude of diagnostic imaging procedures. Modern RCM induce severe and prolonged vasoconstriction within the renal medulla and are directly toxic to the extremely metabolically-active renal epithelium such as PT cells and the TAL. Exposure to RCM and the consequential epithelial damage results in an increase in ROS and oxidative stress, vacuolization of tubular cells, damage to the mitochondria in the form of decrease respiration and ATP production, perturbations in the protein folding capacity of the ER resulting in activation of the UPR and ER stress, and decreased activity of survival kinases and activation of pro-apoptotic stress kinases. The current measures to ensure the prevention of CI-AKI are lacking and considerable research needs to be invested into *in vitro* studies to determine the source of direct RCM induced cytotoxicity.

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## 11. Author Contributions

*D.B. Ward and M.A. Valentovic wrote the manuscript.*

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#### FOOTNOTES:

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### LEGENDS FOR FIGURES

**Figure 1. Relationship between osmolality and viscosity for RCM agents.** Maximum concentrations of iodine provided by the manufactures and corresponding viscosities at 37°C. Refer to Table 1 for values.

**Figure 2. Molecular structures of different RCM.** Parent compound to modern RCM, acetrizoic acid (A), first generation ionic monomer, diatrizoic acid (B), second generation non-ionic monomer, Iohexol (C) and ionic dimer, ioxaglate (D), and third generation non-ionic dimer, iodixanol (E).

**Figure 3. ROS scavenging pathways in renal epithelial cells.**

**Figure 4. Pathways involved in the UPR.**

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**Generations of Radiocontrast Media**

Generation	RCM Name	Osmolality (mOsmol/Kg)	Iodine Content (mg I/mL)	Viscosity 25°C/37°C (cP or mPa.s)	Ionicity	Iodine-to-molecule Ratio
First Generation	Iothalamate	600-1400	141-282	2.0/1.5 - 6.0/4.0	Ionic Monomer	1.5 : 1
	Diatrizoate	1551	370	16.4/10.45		
	Metrizoate	2100	370	-/4		
Second Generation	Ioxaglate	600	320	15.7/7.5	Ionic Dimer	3 : 1
	Ioxilan	721	350	16.3/8.1	Nonionic Monomer	
	Iopromide	328-774	150-370	2.3/1.5 - 22.0/10.0		
	Ioversol	502-792	240-350	4.6/3.0 - 14.3/9.0		
	Iopamidol	524-796	250-370	5.1/3.0 - 20.9/9.4		
	Iohexol	322-844	140-350	2.3/1.5 - 20.4/10.4		
Third Generation	Iodixanol	290	270-320	12.7/6.3 - 26.6/11.8	Nonionic Dimer	6 : 1

**Table 1. Summary of various parameters of RCM.** (American College of Radiology Committee on Drugs and Contrast Media, 2017).

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**Predisposing Risk Factors**

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Preexisting Renal Impairment  
Diabetes Mellitus  
Congestive Heart Failure  
Decreased Effective Intravascular Volume  
Salt Depletion  
Dehydration  
Prolonged Hypotension  
Low Hematocrit  
Age > 70 Years  
Multiple Myeloma  
Sepsis  
Concomitant Use of Various Drugs

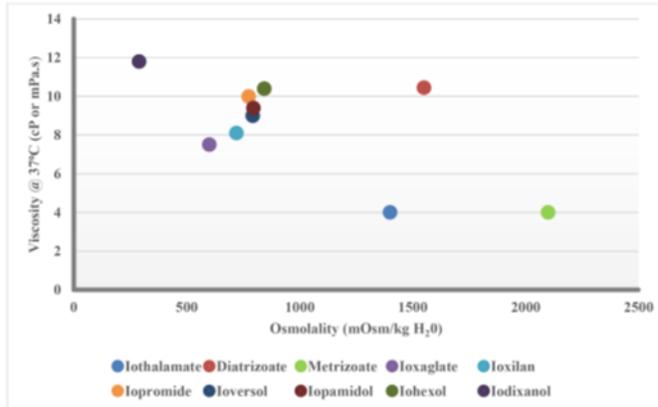
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**Table 2. Common predisposing risk factors for CI-AKI**

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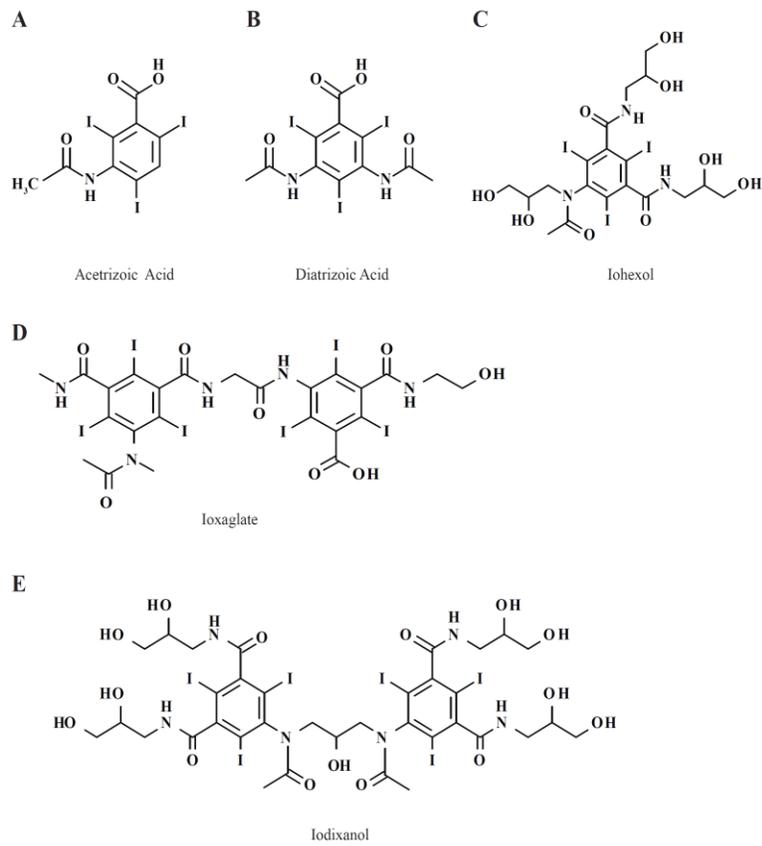
## FIGURES

Figure 1



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**Figure 2**



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**Figure 3**

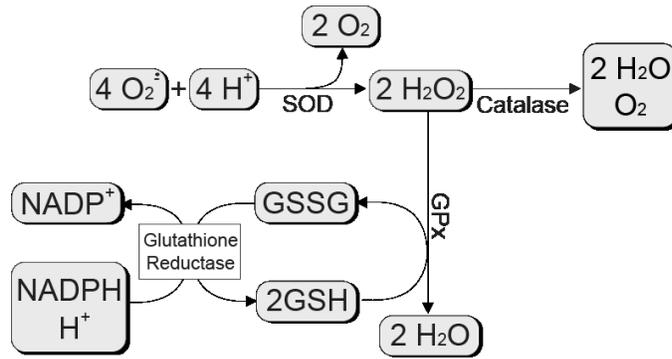


Figure 4

