Clinical and Experimental Evidence for Prevention of Acute Renal Failure Induced by Radiographic Contrast Media

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Received January 31, 2005

Abstract. Acute renal failure still occurs as a complication after radiographic examination using iodinated radiographic contrast medium. The incidence rate of radiographic contrast medium-induced nephropathy (radiocontrast nephropathy) is low (2 – 3%) in general. However, the rate is remarkably elevated in patients with pre-existing renal insufficiency. Radiocontrast nephropathy is associated with increased morbidity and mortality, particularly in patients with percutaneous coronary interventions. Although the reduction in renal blood flow and direct toxic action on renal tubular cells are considered to be involved, little is known about the etiology of radiocontrast nephropathy. A number of agents that improve renal circulation have been clinically tested for prevention of radiocontrast nephropathy, but none of them has succeeded. Protection of renal tubular cells against oxidative stress is another approach to avoid radiocontrast nephropathy. Prophylactic effects of antioxidants such as N-acetylcysteine and ascorbic acid have been reported by several investigators, although the effectiveness of these compounds is still a matter of debate. At present, hydration is regarded as the only effective, though incomplete, prophylactic regimen for radiocontrast nephropathy. Recently, we have shown that caspase-dependent apoptosis is an important factor in the pathogenesis of radiocontrast nephropathy and clarified cellular mechanisms underlying the radiocontrast media-induced apoptosis. This review summarizes clinical and experimental evidence for the etiology and prevention of radiocontrast nephropathy.

Keywords: radiocontrast nephropathy, decrease in renal blood flow, oxidative stress, prophylaxis, apoptosis of renal tubular cells

Introduction

Iodinated radiographic contrast media (RCM) are increasingly used along with recent progress in diagnostic imaging techniques. However, the occurrence of acute renal failure known as radiocontrast nephropathy is still a major complication after radiographic examination. Radiocontrast nephropathy is a matter of serious problem, particularly in patients with reduced renal function. According to the report of the Japanese Ministry of Health and Welfare regarding drug-induced renal failure surveyed during 1989 – 1996, radiocontrast nephropathy occurred in 5 of 234 cases of acute renal failure (2.1%) and is the seventh leading cause of drug-induced renal failure (1). In the United States and Europe, radiocontrast nephropathy is reported to be the third leading cause of acute renal failure accounting for 10% of all causes of hospital-acquired renal failure (2). Radiocontrast nephropathy is associated with elevation of serum creatinine, which appears transiently in many cases within 48 – 72 h after injection of RCM, but it develops occasionally chronic renal failure that requires dialysis. It has recently been reported in 7,230 patients who undertaken percutaneous coronary interventions that patients who developed radiocontrast nephropathy have more frequent clinical adverse events such as myocardial infarction, longer hospital stay, and higher one-year mortality rate than those who showed no signs of nephropathy (3). Therefore, prevention of radiocontrast nephropathy is essentially important in view
of improving compromised quality of life as well as minimizing economical costs.

Although little is known about cellular mechanisms underlying radiocontrast nephropathy, direct toxic action on renal tubular cells (renal mechanism) (4 - 7) and/or decrease in renal blood flow (pre-renal mechanism) (8, 9) have been considered to be involved in the pathogenesis of radiocontrast nephropathy. A number of compounds that improve renal blood flow have been clinically tested for prevention of radiocontrast nephropathy, but most of them were not effective. On the other hand, several recent clinical trials have shown that antioxidant agents such as N-acetylcysteine (NAC) (10, 11) and ascorbic acid (12) are slightly but significantly effective for prevention of radiocontrast nephropathy, thereby suggesting that the oxidative stress rather than the reduction in renal blood flow plays an important role in the development of radiocontrast nephropathy.

In this review, we summarize the data of the representative clinical studies for prevention of radiocontrast nephropathy. We also show our recent experimental findings on the mechanisms and prevention of the renal injury induced by RCM.

Definition, incidence rate, and risk factors of radiocontrast nephropathy

Definition

Although there is no definite hematological criteria for radiocontrast nephropathy, the guidelines on administering RCM provided by the European Society of Urogenital Radiology have shown that the elevation of serum creatinine (SCr) by ≥0.5 mg/dL or ≥25% within 3 days of RCM injection is defined as radiocontrast nephropathy (13). Thus, many investigators have employed either one or both as the criteria of radiocontrast nephropathy.

Incidence rate

The incidence of radiocontrast nephropathy varies depending on the volume or physicochemical properties of RCM used, differences in prophylactic regimens such as hydration, and patients’ risks. Meyrier (14) has summarized the data on the incidence of radiocontrast nephropathy reported during 1975 and 1989 and showed that the rate ranges from 3.7% to 70%, averaging 10.2%.

Risk factors

A number of risk factors for radiocontrast nephropathy have been shown by several investigators. Taliercio et al. (15) have reported that there are 4 major risk factors for radiocontrast nephropathy, including cardiac failure, repeated uses of RCM within 72 h, volume of contrast agents, and insulin-dependent diabetes mellitus, in patients with pre-existing renal failure who undertook angiographic examination. Rich and Crecelius (16) have also shown by a prospective study using 183 aged patients (≥70) who undertook cardiac catheterization that there are 5 risk factors for radiocontrast nephropathy, including high SCr (>1.5 mg/dL), large volume of RCM (>200 mL), low serum albumin (<35 g/L), diabetes mellitus, and low serum sodium (<135 mM), and that the incidence rate of nephropathy is reduced from 11.2% in patients who had one of these risks to 1.2% in those who had no risks. Gussenhoven et al. (17) have reported that the renal dysfunction defined as the increase in SCr by ≥10% appears in 21 of 396 patients (5.7%) who undertook angiographic procedures and that these patients have 2 or more of the risk factors such as pre-existing renal disease, age over 70, hypertension, and volume of RCM exceeding 150 mL. The association of diabetes mellitus with radiocontrast nephropathy is not clear in their study. Among these risk factors, pre-existing renal dysfunction and large volume of RCM are particularly important. Porter (18) has reported that the incidence of radiocontrast nephropathy varies between 2% and 7%, but the rate is elevated by 5- to 10-fold in patients with pre-existing renal insufficiency (SCr >1.5 mg/dL). More recently, Rihal et al. (19) have shown in a large scale study of 7,586 patients who undertook percutaneous transluminal coronary interventions in the Mayo Clinic that the incidence rate of radiocontrast nephropathy defined as the elevation of SCr by ≥0.5 mg/dL is 3.3% (254 patients) as a whole, but much lower in patients with low baseline SCr (2.4% for 0 – 1.1 mg/dL SCr and 2.5% for 1.2 – 1.9 mg/dL SCr) than in those with high baseline SCr (22.4% for 2.0 – 2.9 mg/dL SCr and 30.6% for >3.0 mg/dL SCr). They also showed that diabetes increases the risk of radiocontrast nephropathy in patients with baseline SCr <2.0 mg/dL (3.7% versus 2.0% for 0 – 1.1 mg/dL. SCr, P = 0.005; 4.5% versus 1.9% for 1.2 – 1.9 mg/dL SCr, P<0.001), but not in patients with normal renal function before the procedure.

Reducing the injection volume of RCM should also be considered to decrease the incidence of radiocontrast nephropathy. For this purpose, the following equation [1] by Cigarroa et al. (20) may be useful particularly in high-risk patients:

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\text{Maximum volume of RCM} = 5 \, \text{mL/body weight (kg)} \times \frac{[\text{maximum 300 mL}]}{\text{SCr (mg/dL)} - 1} \quad [1]
\]

This equation was deduced from the data obtained from patients who received radiographic examination during 10 years since 1978. Moreover, the availability of this equation was verified in 115 patients with reduced renal function (SCr ≥1.8 mg/dL), in which the incidence
of radiocontrast nephropathy is significantly \((P<0.001)\) lower in patients who received RCM within the maximum dose (2 of 86 patients, 2.3%) than in those who received a large volume of RCM exceeding the maximum value (6 of 29 patients, 20.7%) (20).

Roles of physicochemical properties of RCM in radiocontrast nephropathy

The development of RCM originated from the use of sodium iodide in radiography in 1918. A number of ionic high osmolar contrast media (HOCM) were synthesized through 1950’s to 1960’s, but the incidence of immediate-type hypersensitivity reactions (HSRs), including rush, urticaria, and sometimes shock, was a serious problem. In the 1970’s and 1980’s, non-ionic low osmolar contrast media (LOCM) have been synthesized to minimize such undesirable adverse reactions (21, 22). The incidence of immediate-type HSRs is further lowered in more recently developed iso-osmolar contrast media (IOCM) such as iotrolan and iodixanol, although there is a drawback that these IOCM are prone to cause delayed adverse reactions (23, 24). HSRs associated with these contrast agents appear to result from the release of mast cell ingredients such as histamine and tryptase (25 – 28). Indeed, a number of RCM stimulate the release of these chemical mediators from isolated mast cells, in which the effects are more marked in ionic RCM than in non-ionic RCM (22, 29, 30). However, the contribution of osmotic pressure to the RCM-induced degranulation of mast cells is a matter of controversy (30 – 32).

Ionicity

It remains unclear whether the incidence of radiocontrast nephropathy differs between ionic and non-ionic RCM. Lower incidence of nephropathy in favor of non-ionic RCM has been reported by several investigators. A large-scale multi-center randomized study comparing the ionic agent amidotrizoate and the non-ionic agent iohexol in 1,196 patients has indicated that the incidence of nephropathy defined as the elevation of SCr by \(\geq 1\) mg/dL at 48 or 72 h after injection is significantly lower in patients assigned to iohexol than in those assigned to amidotrizoate (3% versus 7%, \(P<0.002\)) (33). In contrast, no significant difference in the incidence of nephropathy between amidotrizoate and iopamidol has been reported by Schwab et al. (34) who have shown by a randomized controlled study using 443 patients that the incidence rate of radiocontrast nephropathy is 10.2% in the amidotrizoate group and 8.2% in the iopamidol group.

Osmolality

Then, a question arises whether the incidence of radiocontrast nephropathy differs among HOCM, LOCM, and IOCM. Lautin et al. (35) have reported that LOCM induces nephropathy less frequently than HOCM. However, controversial data have been reported by other investigators (36, 37). A meta-analysis of data from 25 clinical studies showed that the incidence rate of radiocontrast nephropathy is significantly lower in LOCM than in HOCM, in which the odds ratio is 0.5 in patients with pre-existing renal impairment and 0.75 in those with normal renal function in favor of LOCM (38). More recently, a randomized study comparing the incidence of radiocontrast nephropathy between IOCM (iodixanol) and LOCM (iohexol) (NEPHRIC study) in 129 patients with baseline SCr of 1.5 – 3.5 mg/dL has demonstrated that the incidence of nephropathy is significantly lower in iodixanol.injected patients (2 of 64 patients, 3%) than in those who received iohexol (17 of 65 patients, 26%) (39). Taken together, it is assumed that IOCM is less nephrotoxic than LOCM or HOCM. Therefore, the use of IOCM is encouraged in patients with reduced renal function. However, the iso-osmolar agents such as iodixanol and iotrolan are not approved for the use in coronary angiography in Japan.

Prophylaxis of radiocontrast nephropathy by hydration

Hydration is regarded as the only effective prophylactic strategy to reduce the incidence of radiocontrast nephropathy (40), so the procedure is inevitably carried out before and after radiographic examination, particularly in high-risk patients. Although the clinical study regarding the effectiveness of hydration in high-risk patients is limited because of the ethical problem, a randomized controlled study has been performed to compare the effects of fluid infusion and free drinking on the incidence of radiocontrast nephropathy in 53 low-risk patients (mean baseline SCr of 1.2 mg/dL). The study has shown that the incidence of radiocontrast nephropathy defined as the elevation of SCr by \(\geq 0.5\) mg/dL within 48 h after RCM injection is significantly higher in patients assigned to free drinking than in those with hydration for 24 h beginning 12 h before RCM injection [9 of 26 patients without hydration (34.6%) versus only one of 27 patients with hydration (3.7%), \(P = 0.005\)] (41).

Comparison between half-normal saline and saline as infusion

Half-normal saline (0.45%) is prevalently used for hydration, since infusion of a large volume of normal
saline leads to not only afterload to the heart due to the increase in plasma Na⁺ (154 mEq in saline versus 142 mEq in plasma) but also acidosis associated with the decrease in HCO₃⁻ and increase in H⁺ after Cl⁻ overload (154 mEq in saline versus 101 mEq in plasma). On the other hand, Mueller et al. (42) have shown in a large-scale randomized controlled study with 1,620 patients that hydration with normal saline is more effective than that with half-normal saline in reducing the incidence of radiocontrast nephropathy [incidence rate: 6 of 809 patients (0.7%) in normal saline group versus 16 of 811 patients (2.0%) in half-normal saline + 5% glucose group, P = 0.04].

Comparison between saline and sodium bicarbonate as infusion

Recently, Merten et al. (43) have reported the beneficial effect of sodium bicarbonate solution as an infusion to prevent radiocontrast nephropathy by a randomized controlled study using 119 patients with mild renal insufficiency (baseline SCr ≥1.1 mg/dL). They have reported that the incidence of nephropathy is significantly lower in patients assigned to hydration with 154 mEq sodium bicarbonate during 1 h before (3 mL/kg per hour) and 3 h after (1 mL/kg per hour) injection of a non-ionic agent iopamidol than those assigned to hydration with saline in the same dosing regimen [one of 60 patients (1.7%) in sodium bicarbonate group versus 8 of 59 patients (13.6%) in saline group, P = 0.02].

Comparative effects of hemodialysis, hemofiltration, and hemodiafiltration for prevention of radiocontrast nephropathy

Hemodialysis

To prevent radiocontrast nephropathy in high-risk patients, hemodialysis is carried out in some cases. Moon et al. (44) reported in a small number of patients that hemodialysis reduces the incidence of nephropathy by facilitating the clearance of RCM. However, the effectiveness of hemodialysis has not been confirmed by several recent studies (45–48). In particular, Vogt et al. (47) have shown that hemodialysis may worsen the renal function after radiographic examination, in which the incidence rate of radiocontrast nephropathy is 44% in patients with hemodialysis and 35% in those without hemodialysis. Moreover, the incidence of chronic renal failure that requires dialysis is 15% in the hemodialysis group and 5% in the non-hemodialysis group. Therefore, the prophylactic hemodialysis is not recommended because of the lack of evidence for its effectiveness as well as high medical costs.

Hemofiltration and hemodiafiltration

Marenzi et al. (49) have recently shown by a randomized controlled trial enrolled 114 patients with pre-existing renal insufficiency that hemofiltration during 4–8 h before and 18–24 h after injection of RCM is effective in reducing the incidence of radiocontrast nephropathy. The study showed that the incidence of nephropathy defined as the increase in SCr by ≥25% is significantly lower in patients with hemofiltration than in those without hemofiltration (5% hemofiltration group versus 50% in control group, P<0.001). They also have shown in the same study that hemofiltration reduces the incidence of in-hospital clinical events (9% in hemofiltration group versus 52% in control group, P<0.001) as well as the in-hospital mortality (2% in hemofiltration group versus 14% in control group, P = 0.02).

By contrast, Gabutti et al. (50) have shown a lack of preventive effect of continuous hemodiafiltration before and after RCM injection in a randomized controlled study on 26 patients with pre-existing renal insufficiency, in which the incidence of radiocontrast nephropathy is 37.5% in the hemodiafiltration group and 24% in the non-hemodiafiltration group. Therefore, further studies are required to confirm the effectiveness of hemofiltration/hemodiafiltration for the prophylaxis of radiocontrast nephropathy.

Effects of several drugs for prevention of radiocontrast nephropathy

Intravascular injection of RCM leads to ischemia in renal tissues due to the vasoconstriction and the resultant decrease in renal blood flow. The RCM-induced decrease in partial oxygen pressure (PaO₂) is marked in the outer medulla where thick ascending limbs of Henle’s loop exist and PaO₂ depends largely on the activities of electrolytes transport systems located on renal tubular membranes (8, 51). It has been demonstrated that several loop diuretics, including furosemide, reduce oxygen consumption by inhibiting Na⁺/K⁺/Cl⁻ co-transporter (52) The renal blood flow in humans is approximately 1 L/min, the rate of which is about one-fifth of the total cardiac output. This implies that the kidney is one of the organs showing the highest oxygen consumption and thus highly vulnerable to ischemic insults. Based on the assumption that ischemia due to vasoconstriction plays a crucial role in the pathogenesis of radiocontrast nephropathy, a number of investigators have examined the effects of several agents that improve the reduction in renal circulation and/or PaO₂ induced by RCM, including furosemide, endothelin antagonists, atrial natriuretic peptide (ANP), dopamine, fenoldopam,
a dopamine D_1-receptor agonist, calcium channel blockers, and prostaglandin E_1, on the incidence of radiocontrast nephropathy. However, the results on the effects of these agents are rather controversial. The other approach has recently been made to prevent radiocontrast nephropathy, that is the protection of renal tissues against oxidative stress using antioxidants such as NAC and ascorbic acid. We summarize representative experimental and clinical data on the effects of these agents on the incidence of radiocontrast nephropathy.

**Diuretics**

The use of diuretics such as furosemide in addition to hydration had been considered to be effective for prevention of nephropathy, since loop diuretics attenuate the decrease in PaO_2 in the outer medulla of the kidney by inhibiting the electrolyte transporters. It has been reported in young dogs that RCM-induced oliguric renal failure is attenuated by infusion of furosemide and dopamine in combination with hydration (53). The acute renal failure induced by RCM in unilateral nephrectomized rats pretreated with indomethacin is also attenuated by furosemide (54). However, in the clinical setting, diuretic compounds such as furosemide and mannitol may deteriorate the renal dysfunction after injection of RCM. Solomon et al. (55) have shown the effects of furosemide and mannitol in combination with hydration in a randomized placebo-controlled study on 78 patients with pre-existing renal dysfunction (average baseline SCr of 2.1 mg/dL) who underwent coronary angiography. In this study, hydration was performed in all patients with half-normal saline during 24 h (from 12 h before to 12 h after radiographic examination). The incidence of radiocontrast nephropathy defined as the elevation of SCr by $\geq 0.5$ mg/dL was significantly higher in the furosemide- or mannitol-treated group than in the placebo group [10 of 25 patients (40%) in furosemide group, 7 of 25 patients (28%) in mannitol group versus 3 of 28 patients (11%) in placebo group, $P = 0.05$ versus furosemide or mannitol].

The lack of effect of furosemide has also been shown by another randomized controlled study (PRINCE study), in which the effect of furosemide plus dopamine in combination with hydration (half-normal saline) is compared with that of hydration alone in 98 patients with renal deficiency (basal SCr $\geq 1.8$ mg/dL) who underwent coronary angiography (56). The study has shown that the increase in SCr at 48 h after injection of RCM is not different between the two groups (0.48 mg/dL in furosemide plus dopamine group versus 0.51 mg/dL in placebo group, $P = 0.87$). Although the precise reason for the deteriorative effect of furosemide on renal dysfunction is unclear, it may be associated with the vasoconstriction and decrease in renal blood flow, which is mediated by the enhanced renin release/angiotensinII synthesis in response to the action on the macula densa (57, 58). Taken together, furosemide and mannitol is not recommended for the prophylaxis of radiocontrast nephropathy.

**Endothelin antagonists**

A number of studies described in the literature have suggested a role for endothelin in the pathogenesis of radiocontrast nephropathy. At present, three types of endothelin exist, that includes endothelin (ET)-1, ET-2, and ET-3, among which ET-1 has the most potent vasoconstrictive action. ET-1 also causes a potent constriction of renal arteries, which leads to ischemic renal failure (59). On the other hand, endothelin receptors are classified into ETA and ETB, both of which are highly distributed to the renal tissues (60, 61). It has been reported that RCM increase the concentration of endothelin in plasma and urine of rats (62, 63) and dogs (64). In addition, RCM stimulate the release of endothelin from cultured bovine aortic endothelial cells, in which the extent of endothelin release is correlated with the renal toxicity of RCM assessed by the decrease in creatinine clearance and morphologic damage (65). On the other hand, the decrease in renal blood flow and increase in renal vascular resistance induced by intravenous RCM injection in anesthetized dogs pretreated with indomethacin are reversed by the non-selective endothelin antagonist SB 209670 (66). Moreover, the decrease in PaO_2 induced by RCM in the rat outer medulla is reversed by the selective ETA antagonist BQ123 (52). In rats pretreated with indomethacin and a nitric oxide synthase inhibitor N^6-nitro-L-arginine (L-NAME), intravenous injection of RCM causes marked renal injury, as characterized by the elevation of SCr (67), decrease in glomerular filtration rate, and morphologic observations indicating necrosis in medullary tubular thick ascending limbs (68). These actions of RCM are attenuated by selective ETA antagonists such as A-127722 and BMS-182874 (67, 68). These experimental findings strongly suggest a pivotal role for endothelin in the pathogenesis of radiocontrast nephropathy.

Clinical studies have also shown that endothelin concentrations in plasma and urine increase in patients who received a large volume of RCM ($\geq 150$ mL) (69) or in those with renal insufficiency (70) after radiographic examination. Therefore, it is assumable that endothelin released by RCM causes ischemic renal failure due to vasoconstriction.

However, it is quite unexpected that endothelin antagonists are not effective in the clinical setting for
the prophylaxis of radiocontrast nephropathy, as shown by a randomized controlled trial of 158 patients with pre-existing renal deficiency (mean baseline SCr of 2.7 mg/dL) undergoing cardiac angiography (71). The study has shown that SB 209670 is not effective or rather enhances the incidence of radiocontrast nephropathy defined as the elevation of SCr by ≥0.5 mg/dL or ≥25% from baseline (56% in SB 209670 group versus 29% in placebo group, P = 0.002). The average of the increase in SCr at 48 h after angiography is also higher in the SB 209670 group than in the placebo group (0.7 mg/dL versus 0.4 mg/dL, P = 0.002).

Adenosine antagonists

The reduction of renal blood flow induced by RCM in rats is reversed by adenosine antagonists including theophylline and the adenosine A<sub>1</sub>-receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) (72). On the other hand, RCM increase the cellular concentration of adenosine in cultured renal tubular cells, suggesting that adenosine generated from renal tubular cells by RCM induces vasoconstriction and subsequent reduction in renal blood flow (5, 7). Katholi et al. (73) reported that RCM increase the adenosine concentration in human urine. They also showed that oral ingestion of theophylline ameliorates the reduction in creatinine clearance after radiographic examination. Subsequently, several studies have been carried out to confirm the protective effect of adenosine antagonists against radiocontrast nephropathy. Huber et al. (74) have shown in a randomized placebo-controlled trial of 100 patients with chronic renal dysfunction (baseline SCr ≥1.3 mg/dL) that intravenous theophylline injection (200 mg, 30 min before RCM) is effective for prevention of radiocontrast nephropathy. In this study, the incidence of radiocontrast nephropathy defined as the elevation of SCr by ≥0.5 mg/dL within 48 h after radiography is significantly lower in the theophylline group than in the placebo group (4% versus 16%, P = 0.046). Moreover, urinary excretion of N-acetyl-β-D-glucosaminidase (NAG), a lysosomal enzyme localized specifically in tubular cells (75), is significantly (P = 0.034) elevated at 24 h in the placebo group but not in the theophylline group. Similar results were also reported by Erley et al. (76). However, contradictory results on the effect of theophylline have been reported by several investigators. Kramer et al. (77) have shown in a randomized study comparing the effects of theophylline (a bolus intravenous injection at 4 mg/kg and subsequent continuous infusion at 0.25 mg/kg per hour for up to 96 h) with those of saline in 56 patients with normal renal function that the incidence of radiocontrast nephropathy defined as the elevation of SCr by ≥0.4 mg/dL is not different between the two groups (18% in theophylline group versus 14% in saline group). The lack of preventive effect of intravenous aminophylline (200 mg) on radiocontrast nephropathy has also been reported by Shammas et al. (78).

ANP

The injection of RCM significantly increases plasma level of ANP in patients with or without renal insufficiency or diabetes mellitus (69, 79). Rahman et al. (80) have shown in 53 patients with severe renal failure (mean baseline SCr of 5.1 – 5.3 mg/dL) that the infusion of human ANP (0.20 μg/kg per minute intravenously for 24 h or 0.08 μg/kg per minute intraarterially for 8 h) is useful for reducing the rate of deterioration of renal failure that requires dialysis (23% in patients with ANP versus 52% in patients without ANP, P<0.05). The prophylactic effect of ANP on radiocontrast nephropathy was also reported by Weisberg et al. (81) who showed that infusion of ANP reduces the rate of radiocontrast nephropathy defined as the increase in SCr by ≥25% within 48 h in non-diabetic patients but not in diabetic patients. Later, a randomized placebo-controlled study has been carried out to confirm the efficacy of intravenous ANP partial peptide ANP<sub>1-28</sub> (anaritide, 0.01 – 0.1 μg/kg per minute for 30 min before and after RCM) to prevent radiocontrast nephropathy in 247 patients with baseline SCr between 1.5 and 1.8 mg/dL (82). Unfortunately, the preventive effects of ANP was not observed in this study, in which the incidences of radiocontrast nephropathy, defined as the increase in SCr by ≥0.5 mg/dL or by ≥25% from baseline, are 23 – 25% in the ANP-treated patients and 19% in the placebo group.

Dopamine and D<sub>1</sub>-receptor agonist fenoldopam

It has been demonstrated in dogs that RCM-induced reduction in renal blood flow and decrease in glomerular filtration rate are attenuated by the dopamine D<sub>1</sub>-receptor agonist fenoldopam, while they are exaggerated by a D<sub>1</sub>-receptor antagonist SCH 23390, suggesting a role for dopamine D<sub>1</sub> receptor in the pathogenesis of radiocontrast nephropathy (83). A few non-controlled studies with a small number of patients have shown that fenoldopam prevents radiocontrast nephropathy (84, 85). Recently, the effect of fenoldopam on the incidence of radiocontrast nephropathy has been investigated in a multi-institutional randomized placebo-controlled trial (CONTRAST study) of 315 patients with creatinine clearance <60 mL/min (86). The study has shown that the infusion of fenoldopam at 0.05 – 0.1 μg/kg per minute during 30 min before and 12 h after RCM injection does not affect the incidence of radiocontrast...
nephropathy defined as the increase in SCr by ≥25% from baseline within 96 h after radiography (33.6% in fenoldopam group versus 30.1% in placebo group, \( P = 0.61 \)). Moreover, fenoldopam has no influence on long-term clinical events, including one-month survival (2.0% versus 3.8%), development of severe renal failure that requires dialysis (2.6% versus 1.9%), and the rate of re-hospitalization (17.6% versus 19.9%), thereby suggesting that fenoldopam is not useful for the prevention of radiocontrast nephropathy. The lack of effect of fenoldopam to prevent radiocontrast nephropathy has also been reported in another randomized controlled study of 123 patients with baseline SCr >1.6 mg/dL or creatinine clearance <60 mL/min (87).

Calcium channel blockers

Calcium channel blockers such as diltiazem (88) and amloidipine (89) have been shown in rats to inhibit the renal failure induced by RCM, in which the elevation of SCr induced by the high-osmolar agent amidotrizoate in renal failure induced by RCM, in which the elevation of amlodipine (89) have been shown in rats to inhibit the calcium channel blockers

On the other hand, a randomized placebo-controlled study of 35 patients with renal insufficiency has shown that oral ingestion of nitrendipine (20 mg/day for 3 days) is effective for preventing the decrease in glomerular filtration rate (90). In contrast, later studies with nitrendipine (91), felodipine (92), or amloidipine (93) did not confirm the beneficial effects of calcium channel blockers for prevention of radiocontrast nephropathy.

Prostaglandin E₁

The beneficial effect of prostaglandin E₁ for the prophylaxis of radiocontrast nephropathy was reported by Gurkowski et al. (94) who have shown that repeated treatment with the prostaglandin E₁ analog misoprostol (200 μg, 4 times a day starting from 3 days before to 2 days after RCM) significantly attenuates the decrease in creatinine clearance induced by the radiographic examination. Moreover, another randomized placebo-controlled study has also shown in 130 patients with renal dysfunction (baseline SCr ≥1.5 mg/dL) that infusion of prostaglandin E₁ (10 – 40 ng/kg per minute for 6 h) starting from 30 – 60 min before RCM injection significantly diminishes the increase in SCr determined at 48 h after radiographic examination, in which the effect is most marked at a dose of 20 ng/kg per minute (95). Therefore, prostaglandin E₁ may be promising as a prophylactic agent against radiocontrast nephropathy. Further studies are needed to confirm the effectiveness of this agent.

NAC

As mentioned above, the ischemic condition associated with renal vasconstriction is highly assumable to be implicated in the etiology of radiocontrast nephropathy. During ischemia, ATP is severely depleted while the synthesis of hypoxanthine is enhanced in renal tissues. Hypoxanthine is further metabolized by xanthine oxidase during reperfusion into xanthine with a concomitant liberation of cytotoxic oxygen free radicals, leading to renal injury (96). Therefore, the inhibition of the synthesis and/or the action of oxygen free radicals is an effective approach to prevent ischemic renal injury. Based on the assumption that radiocontrast nephropathy is due to ischemic renal failure, several investigators have tested the effects of xanthine oxidase inhibitors or antioxidants for prevention of radiocontrast nephropathy.

Katholi et al. (97) have shown in 39 patients that urinary xanthine concentration increases to approximately 1.6-fold at 48 h after injection of RCM. They also have shown that oral ingestion of the xanthine oxidase inhibitor allopurinol suppresses the increase in urinary xanthine excretion, while causing a slight and not significant inhibition of the decrease in creatinine clearance associated with the contrast agent (decrease by 40% in allopurinol group versus by 79% in placebo group).

On the other hand, Tepel et al. (10) originally found that NAC is potentially useful for prevention of radiocontrast nephropathy. They showed in a randomized placebo-controlled study of 83 patients with mean baseline SCr of 1.3 mg/dL that oral administration of NAC (600 mg twice daily for 3 days) in combination with hydration with half-normal saline before and after radiography significantly reduces the incidence of radiocontrast nephropathy, defined as the elevation of SCr by ≥0.5 mg/dL within 48 h [1 of 41 patients (2%) in the NAC group versus 9 of 42 patients (21%) in the control group, \( P = 0.01 \)]. They also showed that the average of SCr significantly (\( P<0.001 \)) is lowered at 48 h after radiography in NAC group, while it is not changed in the control group. Subsequently, a number of clinical studies have been carried out to confirm the effectiveness of oral NAC as a prophylactic agent for radiocontrast nephropathy. However, the data are controversial. Recently, meta-analysis of the results obtained from these randomized controlled trials has been reported by several investigators (Table 1). These meta-analyses show that NAC is effective in preventing radiocontrast nephropathy, although the results by Pannu et al. (11) analyzing the data from 15 studies indicate that the effect of oral NAC is near the borderline of significant difference (\( P = 0.049 \)).
More recently, a few studies have been performed to determine the effect of intravenous injection of NAC on the incidence of radiocontrast nephropathy. Baker et al. (101) showed in a randomized controlled study comparing the effects of intravenous NAC (infusion of 150 mg during 30 min before and 50 mg during 4 h after RCM) with those of hydration alone in 80 patients with renal insufficiency (RAPID study) that the incidence of nephropathy is significantly lower in the intravenous NAC group [2 of 41 patients (5%) in the NAC group versus 8 of 39 patients (21%) in the hydration alone group, \( P = 0.045 \)]. In contrast, Webb et al. (102) reported that there is no preventive effect of intravenous NAC in combination with hydration by a randomized controlled study. In this study, a bolus intravenous injection of NAC (500 mg immediately before radiography) did not reduce the incidence of radiocontrast nephropathy defined as the increase in SCr by \( 0.5 \text{ mg/dL} \) \( (16 \text{ of 220 patients (7.3%) in the NAC group versus 13 of 227 (5.7%) in the hydration alone group)} \].

It has recently been demonstrated in a randomized placebo-controlled trial of 180 patients who undertook percutaneous coronary interventions that NAC has no influence on long-term clinical events, in spite of a significant reduction in the incidence of radiocontrast nephropathy (103). In this study, oral ingestion of NAC significantly reduced the incidence of radiocontrast nephropathy defined as the increase in SCr by \( 0.5 \text{ mg/dL} \) (3.6% in patients assigned to NAC versus 15.3% in patients assigned to placebo, \( P = 0.02 \)), but NAC treatment did not significantly change the incidence of in-hospital clinical events, including mortality, myocardial infarction, and urgent dialysis [7 of 95 patients (7.4%) assigned to NAC versus 3 of 85 patients (3.5%) assigned to placebo]. The lack of effect of NAC on long-term clinical events casts some doubts about the use of NAC for the prophylaxis of radiocontrast nephropathy in high-risk patients.

Surprisingly, there has been little evidence for the protective effect of NAC against in vivo or in vitro renal injury induced by RCM. Intravenous injection of iohexol causes renal tubular vacuolization in rats, which is not inhibited by NAC (104). Moreover, NAC does not have any influence on the elevation of SCr after injection of an ionic agent iothalamate in rats pretreated with indomethacin and L-NAME, although the antioxidant reverses the decrease in renal blood flow after injection of iothalamate (105). The exposure of cultured renal distal tubular cells (MDCK cells) to amidotrizoate induces apoptosis, which is also unaffected by NAC (6). In contrast, NAC is effective in ameliorating ischemia/reperfusion-induced renal injury in unilateral nephrectomized rats (106). In addition, NAC protects cultured renal tubular cells against hydrogen peroxide-induced cell injury (107). These results, taken together, raise a question about significant contribution of oxidative stress to the toxic action of RCM on renal tubular cells.

**Ascorbic acid**

Spargias et al. (12) have recently shown in a randomized placebo-controlled study of 231 patients with pre-existing renal insufficiency (baseline SCr \( \geq 1.2 \text{ mg/dL} \)) who undertook percutaneous coronary interventions that the incidence of radiocontrast nephropathy can be reduced by oral ingestion of ascorbic acid. In this study, the incidence of radiocontrast nephropathy defined as the elevation of SCr by \( \geq 0.5 \text{ mg/dL} \) or \( \geq 25\% \) from baseline during 2 and 5 days after radiographic examination is significantly lower in patients assigned to ascorbic acid (3 g daily for 3 days starting from one day before RCM) than in those assigned to placebo [11 of 118 patients (9%) versus 23 of 113 patients (20%), \( P = 0.02 \)]. The average of the increase in SCr after RCM injection was also significantly \( (P = 0.049) \) smaller in the ascorbic acid group than in the placebo group.

Although the prophylactic effect of oral administration of ascorbic acid on the incidence of nephropathy associated with RCM is not so marked (odds ratio 0.38, 95% confidence interval 0.17 – 0.85), this medication is safe and also has little pharmacoeconomic problem. Further studies including a large-scale multi-institutional controlled study are required to confirm the beneficial effect of ascorbic acid to prevent radiocontrast nephropathy.

**Possible mechanisms of radiocontrast nephropathy: experimental findings from our recent studies**

Although little is known about cellular mechanisms underlying radiocontrast nephropathy, decrease in renal blood flow (108) and/or direct toxic action on renal tubular cells (7) is currently considered to be implicated in the pathogenesis of radiocontrast nephropathy. As

### Table 1. Meta-analyses of clinical data obtained from randomized controlled trials (RCT) investigating the effect of oral NAC on the incidence of radiocontrast nephropathy

<table>
<thead>
<tr>
<th>No. of RCT</th>
<th>Odds ratio (95% CI)</th>
<th>( P ) value</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>0.435 (0.215 – 0.879)</td>
<td>0.02</td>
<td>Birck et al. (98)</td>
</tr>
<tr>
<td>7</td>
<td>0.37 (0.16 – 0.84)</td>
<td>0.012</td>
<td>Isenbarger et al. (99)</td>
</tr>
<tr>
<td>8</td>
<td>0.41 (0.22 – 0.79)</td>
<td>0.007</td>
<td>Alonso et al. (100)</td>
</tr>
<tr>
<td>15</td>
<td>0.65 (0.43 – 1.00)</td>
<td>0.049</td>
<td>Pannu et al. (11)</td>
</tr>
</tbody>
</table>

CI: confidence interval
mentioned above, a number of agents that improve renal vascular circulation have failed to succeed in producing definite reduction of the incidence of radiocontrast nephropathy. On the other hand, antioxidants such as NAC and ascorbic acid are found to produce a weak but significant protective action against radiocontrast nephropathy. Therefore, it is assumable that direct toxic action on renal tubular cells via oxidative stress rather than the decrease in renal blood flow contributes to the pathogenesis of radiocontrast nephropathy. Drager et al. (109) reported in patients with mild to moderate renal insufficiency that urinary levels of 15-isoprostane F2s, a specific marker of oxidative stress, and urinary α-glutathione-S-transferase protein, a specific marker of proximal tubular injury, increases after radiographic examination. They also showed that NAC reduces the enhancement of both of these markers after the radiographic procedure. Moreover, the contrast agent causes an enhancement of urinary excretion of NAG, a lysosomal enzyme intrinsic to renal tubular cells. It has also been shown that the excretion of urinary NAG increases more sensitively than SCR after injection of RCM in high-risk patients (76). These results, taken together, suggest that reactive oxygen species-mediated renal tubular cell injury is involved in the pathophysiology of radiocontrast nephropathy.

A variety of RCM have been shown to induce injury in cultured renal tubular cells, as characterized by cell shrinkage and nuclear fragmentation, indicating the apoptotic nature of the cell injury (4 – 7). However, there has been little experimental evidence suggesting the role of reactive oxygen species in renal tubular cell apoptosis induced by RCM. Hizoh and Haller (6) have reported that the DNA damage induced in MDCK cells by the ionic medium amido trizolate is not attenuated by NAC, although NAC has been shown to prevent apoptosis induced by hydrogen peroxide in cultured renal tubular cells (107) and to attenuate ischemia/reperfusion-induced renal injury in unilateral nephrectomized rats (106).

Apoptosis induced by RCM in cultured renal tubular cells

We recently showed that a variety of RCM reduce the cell viability in a porcine renal tubular cell line, LLC-PK1 cells (110). A transient (30 min) exposure of LLC-PK1 cells to a variety of RCM (100 mg iodine/mL), including ionic agents ioxaglate, amido trizolate, and iothalamate and non-ionic agents ioversol, io hexol, iopamidol, iomeprol, and iotrolan, induces the loss of cell viability 24 h later as assessed by the mitochondrial enzyme activity with WST-8. The RCM-induced cell injury is associated with the increase in the number of cells stained with fluorescent isothiocyanate-labeled Annexin V, which binds specifically to phosphatidyl serine that translocates from the inside of membrane to the outside during the early stage of apoptosis (111), appearance of terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL)-positive cells, and DNA fragmentation as observed by DNA electrophoresis (Fig. 1). Moreover, the activation of caspase-3 and caspase-9 but not caspase-8 is observed after exposure to RCM. Several inhibitors of caspase-3 and caspase-9 are found to reverse the cell injury and nuclear fragmentation induced by RCM. These findings suggest that RCM-induced injury of renal tubular cells is mediated predominantly by caspase-dependent apoptosis. The extent of the cell injury is different between ionic and non-ionic RCM. Moreover, it is unlikely that the hyper-osmolality contributes to the nephrotoxic actions of RCM, since hyper-osmolar mannitol solution does not mimic the action of RCM. Moreover, there is no significant relationship (r = −0.652) between the extent of cell injury and osmolality of RCM solutions (110).

On the other hand, the changes in apoptosis-related Bcl-2 family proteins may initiate the RCM-induced apoptosis since the non-ionic agent ioversol causes marked reductions in both mRNA expression and protein content of Bcl-2, an apoptosis-inhibitory protein, with concomitant increases in the mRNA expression and protein content of Bax, an apoptosis-facilitatory protein (110, 112). The up-regulation of Bax and/or down-regulation of Bcl-2 depolarizes mitochondrial membrane to stimulate the release of cytochrome c (113 – 115), which in turn, binds to the adapter molecule apoptotic protease activating factor-1 (Apaf-1) and degrades procaspase-9 into caspase-9, an activated form (116). The activated caspase-9 triggers the conversion of pro-caspase-3 into activated caspase-3, leading to the chromosomal DNA fragmentation and cellular morphology changes characteristic of apoptosis (117, 118). Then, a question arises about how RCM changes the expression for Bcl-2 and Bax. We have recently found that ioversol inhibits cyclic AMP-stimulated phosphorylation of Akt, a serine/threonine kinase that is involved in cell survival and differentiation (119), followed by inhibition of phosphorylation of cyclic AMP response element binding protein (CREB) (112) (Fig. 1). It has been demonstrated that phosphorylated CREB binds to the CRE site located on the promoter region of the bcl-2 gene and up-regulates Bcl-2 expression (120 – 122). Therefore, RCM-induced reduction in Bcl-2 expression may result at least in part from inhibition of CREB phosphorylation. On the other hand, phosphorylation of Akt is reported to stimulate CREB activity and enhance Bcl-2 expression (123). Taken together, it is suggested...
Apoptotic injury induced by the non-ionic contrast medium ioversol in LLC-PK1 cells. Ioversol causes cell injury as measured by propidium iodide (PI) staining, while it induces apoptosis as assessed by fluorescent isothiocyanate-labeled Annexin V and terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) stains. Ioversol-induced DNA fragmentation assessed by DNA electrophoresis is dependent on caspase-3 and caspase-9 but not caspase-8 (lane 1: no treatment; lane 2: ioversol; lane 3: + zVAD-fmk, a pan-caspase inhibitor; lane 4: + zDEVD-fmk, a caspase-3 inhibitor; lane 5: + zIETD-fmk, a caspase-8 inhibitor; lane 6: + zLEHD-fmk, a caspase-9 inhibitor). Ioversol also causes mitochondrial membrane depolarization as detected by staining with JC-1, which may result from changes in mRNA expression for Bcl-2 family proteins such as Bcl-2 and Bax as assessed by RT-PCR. The reduction in phosphorylated Akt (pAkt) followed by decrease in the phosphorylated form of cyclic AMP response element binding protein (pCREB) may contribute to the ioversol-induced apoptosis in renal tubular cells. Data partly from Yano et al. (Refs. 109 and 123).
that RCM induce caspase-dependent apoptosis in renal tubular cells by reducing the expression for Bcl-2, which may be attributable to the inhibition of phosphorylation of Akt. On the other hand, NAC was not effective in suppressing these cellular events and apoptosis induced by RCM in LLC-PK1 cells, thereby suggesting a lack of involvement of reactive oxygen species in our in vitro RCM-induced renal injury model (unpublished observations).

Protection by a prostacyclin analog beraprost against renal tubular injury induced by RCM

We reported earlier that ioversol-induced apoptotic injury in LLC-PK1 cells is attenuated by dibutyryl cyclic AMP (DBcAMP) (110, 124). The ioversol-induced cellular events, including reduction in the phosphorylation of Akt and CREB, changes in the expression for Bcl-2 family proteins, activation of caspase-3 and caspase-9, and DNA fragmentation, are all inhibited by DBcAMP. The protective effect of DBcAMP is reversed by H89, an inhibitor of protein kinase A, suggesting an involvement of protein kinase A. Interestingly, the inhibitory effects of DBcAMP on ioversol-induced cell injury and cellular events are abolished by the phosphatidylinositol (PI) 3-kinase inhibitor wortmannin and Akt inhibitor SH-6 (110, 112). Moreover, DBcAMP is no longer effective in reversing the ioversol-induced cell injury in LLC-PK1 cells transfected with a dominant-negative form of CREB (ProCREB) (112). Therefore, it is suggested that cyclic AMP attenuates RCM-induced apoptosis of renal tubular cells by enhancing Bcl-2 expression through activation of protein kinase A/PI 3-kinase/Akt/CREB pathways.

Prostacyclin (IP) receptor is coupled to the stimulatory G protein (Gs) to enhance cAMP production (125). Both the immunohistochemical study using polyclonal antibody raised against IP receptor and in situ hybridization have demonstrated that IP receptor distributes to the glomerular cells, endothelial cells, distal tubules, and collecting ducts in human kidneys (126). Prostacyclin is reported to attenuate the diabetic renal injury induced by streptozotocin in unilateral nephrectomized rats (127) and ischemia/reperfusion-induced renal injury in dogs (128). Although the protective effect of prostacyclin is considered to be due primarily to the vasodilatory action, direct protective action on renal epithelial cells is also shown in the in vitro model of hypoxia/reoxygenation-induced injury in cultured rat proximal renal tubular cells (129). However, it is unknown whether prostacyclin reduces RCM-induced renal failure. In our previous report (124), beraprost at concentrations ranging from 10 nM to 1000 nM attenuates the ioversol-induced decrease in the viability of LLC-PK1 cells in a concentration-dependent manner, in which the protective effect of beraprost is dependent on the elevation of cellular cyclic AMP content and activation of protein kinase A. Beraprost also stimulates the phosphorylation of CREB in a manner dependent on the activation of protein kinase A. Moreover, the prostacyclin analog reverses the ioversol-induced changes in Bcl-2 family proteins and activation of caspase-3 and caspase-9.

Prophylactic effect of beraprost in an experimental model of radiocontrast nephropathy

In mice with unilateral renal occlusion, intravenous injection of ioversol (4 g iodine/kg) causes a marked increase in urinary excretion of NAG determined at 24 h after injection (112). Histological observations show that some TUNEL-positive cells appear in the tubular and interstitial cells in the outer medulla of the kidney. The activity of caspase-3 in this region is also remarkably elevated. Moreover, the expression for Bcl-2 dramatically reduced, while that for Bax increased, in the renal medulla after injection of ioversol. These findings suggest that acute renal failure induced by intravenous injection of ioversol in mice with unilateral renal occlusion is mediated by caspase-dependent tubular apoptosis associated with down-regulation of Bcl-2 and up-regulation of Bax, which is quite similar to the mechanisms underlying RCM-induced apoptosis in cultured renal tubular cells, as described above. A single intraperitoneal administration of beraprost (0.1 – 0.3 mg/kg) attenuates the ioversol-induced increase in urinary excretion of NAG in mice with unilateral renal occlusion. Moreover, beraprost inhibits almost completely the activation of caspase-3, down-regulation of Bcl-2 and up-regulation of Bax, and the appearance of TUNEL-positive cells induced by ioversol (112). Figure 2 shows the possible mechanisms of the prophylactic effect of beraprost against ioversol-induced apoptotic injury of renal tubular cells.

In addition to having the direct protective action on renal tubular cells described above, beraprost increases renal blood flow by dilating renal arteries (130). Moreover, this compound has a potent anti-platelet action (131). It has been reported that RCM have significant actions on blood coagulation/fibrinolytic activity. Kielpinska et al. (132) have shown that the non-ionic agent iopromide is more prone to decrease fibrinolytic activity than the ionic agent ioxaglate, particularly in patients undergoing renal angiography, and they consider that ionic RCM should be used during angiographic procedures in patients with high-risk for thrombotic complications. In addition, Van Beek et al. (133) reported in 14 patients that iohexol is more likely to induce platelet activation than ioxaglate. Therefore,
the anticoagulant action associated with beraprost may be useful in patients with ischemic cardiovascular diseases who undertook angiographic examination with non-ionic RCM.

Taken together with these findings, it is highly assumable that beraprost is potentially useful for the prophylaxis of radiocontrast nephropathy. We are now undergoing a clinical study on the effect of beraprost for prevention of radiocontrast nephropathy in patients with pre-existing renal insufficiency who undertake coronary angiography.

Concluding remarks

Radiocontrast nephropathy is associated with increased morbidity and mortality, particularly in high-risk patients who have undertaken coronary angiography and/or percutaneous coronary interventions. Unfortunately, there have hitherto been few effective regimens for prevention of radiocontrast nephropathy. Hydration with half-normal saline, normal saline, or sodium bicarbonate solution is regarded as the only effective and reliable prophylactic treatment, although the preventive effect is not sufficient in high-risk patients. The lack of more effective prophylactic treatment may be due to the obscurity of the etiology of radiocontrast nephropathy. Although it is postulated that the impairment of renal circulation as well as the direct tubular injury are implicated in the pathogenesis of radiocontrast nephropathy, the former mechanism is least probable because of the presence of a number of negative results regarding the clinical effects of agents that improve renal blood flow for prevention of radiocontrast nephropathy. In contrast, somewhat beneficial clinical effects of antioxidants such as NAC and ascorbic acid may shed light on the enigmatic incidence of radiocontrast nephropathy. Moreover, our recent experimental findings suggest a crucial role for direct tubular damage due to apoptosis in the pathogenesis of radiocontrast nephropathy. In this respect, the development of agents focusing on the interruption of intracellular signals that lead to apoptosis of renal tubular cells after exposure to RCM may become a breakthrough for prevention of radiocontrast nephropathy.

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