Effects of Agmatine on Contrast-Induced Nephropathy in Rats and Rabbits

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Renal insufficiency secondary to contrast administration remains a prevalent and debilitating complication of angiographic procedures. Contrast-induced nephropathy (CIN) is a common clinical problem for which there is no effective medical treatment. However, agmatine has been shown to be effective against ischemia/reperfusion-induced acute kidney injury in rats, a similar condition to CIN. Our aim was to examine the protective effects of agmatine in a rat model of CIN and, based on those results, in a rabbit model of CIN. CIN in the rat model was induced by intravenous administration of indomethacin (10 mg/kg), Nω-nitro-L-arginine methyl ester (L-NAME) (10 mg/kg) and iopamidol (OYPALOMIN, 7.4 g iodine/kg) at 2 weeks after a unilateral nephrectomy. CIN in the rabbit model was induced by intrarenal arterial injection of only iopamidol (BYSTAGE, 4.8 g iodine/kg). Intravenous injection of agmatine (0.1 and 0.3 mmol/kg) did not attenuate the CIN-induced renal insufficiency in the rat model. Intravenous injection of agmatine (0.3 mmol/kg) attenuated the CIN-induced renal insufficiency in the rabbit model such as increases in blood urea nitrogen and plasma creatinine levels. Renal histological damage was also improved by the agmatine administration. The difference in effects of agmatine injection between CIN rats and CIN rabbits was caused by indomethacin and L-NAME administrations. These results indicate that agmatine prevents the development of CIN-induced renal insufficiency in rabbits, and the effect is accompanied by activation of nitric oxide synthase and subsequent increase of blood flow.

Key words agmatine; contrast-induced nephropathy; intrarenal arterial injection; iopamidol; rabbit

INTRODUCTION

The rapid degradation in kidney function caused by contrast media usually is temporary, but it can result in chronic kidney disease (CKD) or even end-stage renal disease (ESRD). Contrast-induced nephropathy (CIN) is a clinical condition in which serum creatinine is increased by 0.5 mg/dL (or more) or by 25% (or higher) within 3 d of contrast use, and is still the third primary cause of hospital-acquired acute kidney injury (AKI). Although the use of contrast media is indispensable in several diagnostic imaging techniques, strategies to overcome CIN are still insufficient,13 which can lead to an increase in length of hospital stay, morbidity, and mortality. Therefore, novel therapeutic strategies for the overcoming of CIN are required.

It is well-known that the causes of CIN are multifactorial, but the precise pathophysiologic mechanisms are not yet fully comprehended. However, vasoconstriction, oxidative stress, and hypoxia constitute the main responsible pathways of CIN.12 In studies which focused on reactive oxygen species, ascorbic acid (vitamin C), α-tocopherol (vitamin E), and N-acetylcysteine were examined to prevent CIN in clinical trials1–6 and animal experiments.7–8 However, contradictory results have been found in several trials, which have led to the search for other candidate renoprotective compounds.

Agmatine is a polyamine found in mammals, which is made by decarboxylation of L-arginine in mammalian tissues.9 We found that agmatine efficiently attenuated renal insufficiency and degeneration induced by ischemia/reperfusion (I/R), which is a condition of AKI.10 Moreover, we reported that agmatine could suppress the increased renal sympathetic nerve activity (RSNA) during renal ischemia via the activation of imidazoline I1 receptors and the excessive outflow of renal norepinephrine after the I/R through the activation of α2 or I1 receptors.11 Alpha2-adrenergic agonists prevent CIN in mice by preserving outer medullary renal blood flow.12 However, it has remained unclear whether agmatine could have a renoprotective effect against CIN.

The aim of the current study was to evaluate the renoprotective effect of agmatine against CIN.

MATERIALS AND METHODS

Animals Seventeen-week-old male Sprague-Dawley rats (Charles River Laboratories Japan, Inc., Yokohama, Japan) and 15-week-old male JW rabbits (Kitayama Labes Co., Ltd., Ina, Japan) were used. The animals were kept in an air-conditioned room (20–26°C) under a 12-h light–dark cycle and were allowed ad libitum access to food and water. This study was carried out according to the guidelines (Nihon Bioresearch Inc., Animal Experiment Ethics Committee) concerning animal experiments and the protection and management of animals maintained at experimental institutions.

Reagents and Materials Agmatine, indomethacin and Nω-nitro-L-arginine methyl ester (L-NAME) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Beraprost was purchased from Cayman Chemical (Ann Arbor, MI, U.S.A.). OYPALOMIN was purchased from Fuji Pharma (Tokyo, Japan). BYSTAGE was purchased from Teva Takeda Pharma (Aichi, Japan). Polyethylene catheter (Inframedic...
PE-50) was purchased from Becton, Dickinson and Company (Franklin Lakes, New Jersey, U.S.A.). Angiographic catheter (GOODTEC ANGIOGRAPHIC CATHETER) was purchased from GOODMAN (Aichi, Japan).

Induction of Contrast-Induced Nephropathy in Rabbits

Two weeks before the radiocontrast administration (at 15-week-old), the right kidney was removed through a small side cut under isoflurane anesthesia (1–3%). After a 2-week recovery period, to induce CIN, the rats were anesthetized with pentobarbital (45 mg/kg, intraperitoneal (i.p.)) and butorphanol tartrate (1 mg/kg, subcutaneous (s.c.)). Polyethylene catheters (Intramedic PE-50) filled with physiological saline containing 20 units/mL of sodium heparin were placed in the femoral veins on the right or left hind limbs. After prior blocking of prostaglandin and nitric oxide (NO) synthesis, rats were injected intravenously with the low-osmolar monomeric iodinated radiocontrast medium iopamidol (OYPALOMIN, 7.4 g iodine/kg). For blocking of cyclooxygenase and NO synthase, rats were injected with indomethacin (10 mg/kg intravenous (i.v.), dissolved in phosphate buffered saline (PBS) (pH 8.4)) and l-NAME (10 mg/kg i.v., dissolved in physiological saline), respectively, 30 min before iopamidol administration.

Animal Groups and Experimental Design in Rabbits Rats were grouped into sham-operated group, vehicle-treated CIN group (control group), and drug-treated CIN groups. Agmatine (0.1 and 0.3 mmol/kg, dissolved in physiological saline) or vehicle (physiological saline) was administered as a gentle bolus injection into a polyethylene catheter placed in the femoral vein (1 mL/kg) 5 min before iopamidol injection. Beraprost (0.1 mg/kg, positive control, dissolved in PBS (--) was administered subcutaneously (1 mL/kg) 5 min before iopamidol injection. At 1 d after iopamidol injection, whole blood was collected from the thoracic aorta, and then animals were euthanized by exsanguination from the abdominal aorta under isoflurane anesthesia.

Induction of Contrast-Induced Nephropathy in Rabbits To induce CIN, 15-week-old rabbits were anesthetized with isoflurane (1–3%) and lidocaine (40 mg/rabbit, s.c.). Angiographic catheters (GOODTEC ANGIOGRAPHIC CATHETER) filled with physiological saline containing 20 units/mL of sodium heparin were placed in the carotid artery. The rabbits were injected with the low-osmolar monomeric iodinated radiopaque medium iopamidol (BYSTAGE, 4.8 g iodine/kg) into the intrarenal artery through the carotid artery. BYSTAGE was chosen for the reason that it had the same active ingredient, additive content, pH, and osmolar ratio as OYPALOMIN. During the procedure the location of the injection was confirmed with Surgical X-ray apparatus (ARCADIS Avantic, Siemens Healthcare, Erlangen, Germany). The other rabbits were injected intravenously with the low-osmolar monomeric iodinated contrast medium iopamidol (BYSTAGE, 4.8 g iodine/kg) through the pinna vein.

Animal Groups and Experimental Design in Rabbits In the preliminary study (preparation of CIN), rabbits were grouped into intravenous-induced CIN group (16 mL/kg), and intrarenal artery-induced CIN groups (16 mL/kg (unilateral 8 mL/kg), 120 and 240 mL/h). Blood samples were taken from the pinna vein before (pre), and 1, 2, 3, 4, 7, and 14 d after iopamidol injection. In the main study, rabbits were grouped into intrarenal artery-induced CIN group (vehicle-treated control group, 16 mL/kg (unilateral 8 mL/kg), 120 mL/h), and agmatine (0.1 and 0.3 mmol/kg) treated CIN group. Agmatine or vehicle (physiological saline) was administered as a gentle bolus injection into the pinna vein (1 mL/kg) 5 min before iopamidol injection. Blood samples were taken from the pinna vein before (pre), and 1 and 2 d after iopamidol injection. The kidneys were excised at the end of blood sampling 2 d after iopamidol injection and examined using a light microscope. Then animals were euthanized by exsanguination from the abdominal aorta under isoflurane anesthesia.

Blood Biochemical Examination The collected blood was centrifuged, and the obtained plasma was used for measurement of renal functional parameters. Blood urea nitrogen (BUN) and creatinine in plasma (Cre) were determined using Automatic Biochemical Analyzer (AU400, Beckman Coulter, CA, U.S.A.).

Histopathological Examination In the main study of rabbits, excised kidneys were processed for histopathological examination, according to standard procedures. The kidneys were then preserved in phosphate-buffered 10% formalin, after which they were made into small pieces, embedded in paraffin wax, sliced to 4 μm, and stained with hematoxylin and eosin. Histopathological alterations were analyzed for proteinaceous casts, medullary congestion, and tubular necrosis, as described by Caramelo et al.13 Proteinaceous casts and tubular necrosis were graded as follows: no damage (0), mild (1; unicellular, patchy isolated damage), moderate (2; damage less than 25%), severe (3; damage between 25 and 50%), and very severe (4; more than 50% damage). The degree of medullary congestion was graded as no congestion (0), mild (1; vascular congestion with identification of erythrocytes by ×400 magnification), moderate (2; vascular congestion with identification of erythrocytes by ×200 magnification), severe (3; vascular congestion with identification of erythrocytes by ×100 magnification), and very severe (4; vascular congestion with identification of erythrocytes by ×40 magnification). The scoring of the histopathological data was assigned by independent observers in a double-blind manner.

Statistical Analysis All numerical values are expressed as means ± standard error of the mean (S.E.M.). Relevant data were processed by Instat (Graph-PAD Software for Science). For within-group data (preliminary study in rabbits), we used one-way repeated measures ANOVA followed by Dunnett’s multiple range test. For among-group data, we used one-way ANOVA followed by Tukey’s multiple comparison test (study in rats) or Dunnett’s multiple comparison test (main study in rabbits). For histopathological data, we used Kruskal–Wallis nonparametric test combined with a Steel-type multiple comparison test. In all comparisons, a significance level was 5%, and probabilities are shown as p < 0.05 or p < 0.01.

RESULTS

Effects of Treatment with Agmatine on Contrast-Induced Nephropathy in Rabbits First, we examined BUN and Cre of the CIN rats subjected to agmatine or beraprost administration. It has been reported that beraprost, a prostacyclin analogue, has a renal protective effect on CIN model.14 Hence, we administered beraprost as a positive control to CIN rats. As shown in Fig. 1, the renal function of control rats showed marked aggravation when measured at 1 d after iopamidol injection. Compared with sham rats, CIN rats showed
significant increases in BUN and Cre. Injection of agmatine (0.1 and 0.3 mmol/kg) to CIN rats did not attenuate the iopamidol induced renal dysfunction.

However, injection of beraprost to CIN rats attenuated the iopamidol induced renal dysfunction.

Preparation of Contrast-Induced Nephropathy in Rabbits

Next, we developed a CIN model using rabbits and investigated the effect of agmatine. In order to cause CIN, contrast medium was given in the vein or renal artery. Surgical X-ray apparatus (ARCADIS Avantic) was used to confirm the injection site (Fig. 2). As shown in Fig. 3, the renal function of rabbits subjected to iopamidol injection into the intrarenal artery (120 and 240 mL/h) showed marked deterioration when measured at 1, 2, 3, and 4 d after iopamidol injection, while the intravenous injection did not show increases in Cre at 1, 2, and 3 d after iopamidol injection. Compared with renal function before radiocontrast administration, CIN rabbits (intrarenal artery) showed flow rate-dependent increases in BUN and Cre. However, two of five rabbits subjected to iopamidol injection into the intrarenal artery at the higher dosage rate (16 mL/kg, 240 mL/h) died during the contrast medium administration (the other rabbits did not die in this study). Therefore, in the main study, we administrated iopamidol into the intrarenal artery at the lower dosage rate (16 mL/kg, 120 mL/h).

Effects of Treatment with Agmatine on Contrast-Induced Nephropathy in Rabbits

As shown in Fig. 4, the renal function of rabbits subjected to iopamidol injection into the intrarenal artery showed marked deterioration when measured at 1 and 2 d after iopamidol injection. Injection of agmatine (0.3 mmol/kg) to CIN rabbits attenuated the iopamidol induced renal dysfunction at 2 d after iopamidol injection. Moreover, injection of beraprost (0.3 mg/kg, s.c.) attenuated the renal dysfunction in CIN rabbits at 2 d after iopamidol injection (BUN: 19.0 ± 4.1 mg/dL, Cre: 1.13 ± 0.35 mg/dL, n = 3, data not shown). Histopathological examination disclosed severe findings in the kidney of CIN rabbits at 2 d after iopamidol injection. These findings were characterized by proteinaceous casts in tubuli in the inner zone of medulla (Figs. 5B, D), medullary congestion, and hemorrhage in the outer zone inner stripe of medulla (Figs. 5G, I), and tubular necrosis in the outer zone outer stripe of medulla (Figs. 5L, N). Injection of agmatine (0.3 mmol/kg) significantly attenuated the proteinaceous casts in tubuli (Figs. 5C, E, 6A).
DISCUSSION

First, we examined the effects of treatment with agmatine on CIN rats. We found that treatment with agmatine (0.1 and 0.3 mmol/kg) did not affect renal dysfunction of CIN rats. The agmatine doses were chosen as they showed renoprotective effects against ischemic acute kidney injury in rats in previous studies. The rat kidney structure is different from human. Anatomically, rodents generally have a one-papilla kidney compared to the multi-papilla human kidneys. Number of nephrons, concentrating ability and glomerular diameter in rodent kidney are different from these in human kidney. It has been reported that administration of contrast media alone induces little or no renal dysfunction in rats. To develop CIN rats, we administrated indomethacin, a cyclooxygenase inhibitor, and l-NAME, a non-selective nitric oxide synthase inhibitor, before the contrast medium injection. However, it has been reported that the cytoprotective effect of agmatine is inhibited by the administration of indomethacin or l-NAME. It is well known that agmatine leads to induction of endothelial NO synthase (eNOS) and consequent increased NO levels, and results in vasodilation. In the current study, effects of treatment with agmatine on CIN rats might have been counteracted by the administrations of indomethacin and l-NAME. Therefore, we needed a CIN animal model that did not require indomethacin and l-NAME.

Experience suggests that intravenous injection of contrast medium to rabbits rarely causes abnormal findings of kidney. It has been reported that intravenous administration of contrast medium alone to rabbits increases serum creatinine and causes severe histological changes in the kidney. Thus, it was assumed that rabbit kidneys were more sensitive to contrast medium than other animals (rats, dogs etc.), and we developed a CIN model using rabbits. Results clearly indicated that intrarenal arterial injection of contrast medium caused renal dysfunction and histopathological changes. Moreover, intravenous injection of agmatine (0.3 mmol/kg) to CIN rabbits attenuated the contrast-induced renal dysfunction, although intravenous injection of the same dose of agmatine to CIN rats did not attenuate it. Agmon et al. demonstrated that the radiocontrast agent reduced blood flow in the kidney cortex of rat and also slightly reduced creatinine clearance. Through activation of NO synthase, l-arginine prevents the decrease in glomerular filtration rate and renal blood flow in CIN to hypercholesterolemic rats, and attenuates the decrease in inulin clearance after radiocontrast agent administration to salt-depleted rats. Thus, it is reasonable to consider that agmatine attenuates the contrast-induced renal dysfunction through the activation of NO synthase. We have not investigated NOx concentrations, effect of NO donor or effect of combined use of agmatine and l-NAME on CIN rabbits, therefore, further studies will be needed. Meanwhile, we have reported that

![Fig. 3. Time Course of Blood Urea Nitrogen (BUN) (A), and Plasma Creatinine (Cre) (B) in Rabbits Subjected to the Low-Osmolar Monomeric Iodinated Radiocontrast Medium Iopamidol Injection](image)

Each point and bar represent the mean ± S.E.M. (Intravenous 16 mL/kg: N = 4, Intrarenal artery-induced 16 mL/kg, 120 mL/h: N = 5, Intrarenal artery-induced 16 mL/kg, 240 mL/h: N = 3). *p < 0.05, **p < 0.01 vs. pre (before radiocontrast administration).

![Fig. 4. Effects of Agmatine on Contrast-Induced Nephropathy in Rabbits](image)

Renal function parameters are blood urea nitrogen (BUN) (A), and plasma creatinine (Cre) (B) at pre (before radiocontrast administration), 1, and 2 d after iopamidol injection. Each column and bar represent the mean ± S.E.M. (n = 6). *p < 0.05 vs. control.
agmatine can suppress the excessive outflow of renal norepinephrine after the I/R.\textsuperscript{11} It has been reported that the cause of CIN is vasoconstriction by increases of adenosine and endothelin.\textsuperscript{23} It is assumed that vasoconstriction by an increase of norepinephrine is also the cause of CIN. Further studies are needed to identify whether renoprotective effect of agmatine on CIN rabbits is due to a decrease of norepinephrine.

In our CIN rabbit model, the same amount of contrast medium was administrated at the same rate into arteries of the right and left kidneys (contrast medium was always administered into the right kidney and then administered into the left kidney). Interestingly, the left kidney weight after the
contrast medium administration was heavier and the pathological tissue disorder of the left kidney was severe, compared to the right kidney after the contrast medium administration at 2 d after iopamidol injection (left kidney weight of normal rabbit: 2.6 ± 0.1 g/kg, right kidney weight of normal rabbit: 2.6 ± 0.1 g/kg, left kidney weight of control: 4.8 ± 0.1 g/kg, right kidney weight of control: 3.4 ± 0.1 g/kg, data not shown). Although it is known that the position of the kidneys is different on the right and left, details of the difference in sensitivity of drugs and chemicals in the right and left kidneys are not known. In this study, when confirming X-ray image after the contrast medium administration, the diffusion state of the contrast medium was always different between the right and left kidneys. We suspect that vessel variation or rate of blood flow is different between the right and left kidneys of rabbits and this may be true for humans.

Our results indicate that agmatine has a renoprotective effect on CIN in rabbits. We believe that this effect is caused by activation of NO synthase and subsequent increase of blood flow. Furthermore, agmatine is safe as a medicine because it is an endogenous biological active substance.

Conflict of Interest T. Sugiura, Y. Hirasawa, and T. Toyoshi are employees of Nihon Bioresearch Inc. The study was funded by Nihon Bioresearch Inc.

REFERENCES

1) Andreucci M, Faga T, Pisani A, Sabbatini M, Michael A. Acute kidney injury by radiographic contrast media: pathogenesis and prevention. Biomedi. Res. Int., 2014, 362725 (2014).

2) Andreucci M, Solomon R, Tasanarong A. Side effects of radiographic contrast media: pathogenesis, risk factors, and prevention. Biomedi. Res. Int., 2014, 741018 (2014).

3) Richter SK, Crammage AJ. Evaluation of N-acetylcysteine for the prevention of contrast-induced nephropathy. J. Community. Hosp. Intern. Med. Perspect., 5, 27297 (2015).

4) Kitzler TM, Jaberi A, Sendhofer G, Rehak P, Binder C, Petnehazy T. N-acetylcysteine and prostanoids protect the renal outer medulla from radiocontrast nephropathy via phosphorylation of cyclic AMP response element binding protein. Am. J. Pathol., 166, 1333–1342 (2005).

5) Kiss N, Hamar P. Histopathological evaluation of contrast-induced acute kidney injury rodent models. Biomedi. Res. Int., 2016, 3763250 (2016).

16) Agmon Y, Peleg H, Greenfeld Z, Rosen S, Brezis M. Nitric oxide and prostanooids protect the renal outer medulla from radiocontrast toxicity in the rat. J. Clin. Invest., 94, 1069–1075 (1994).

17) Yokomaku Y, Sugimoto T, Kume S, Araki S, Ishikawa K, Chin-Kanasaki M, Sakaguchi M, Nitta N, Haneda M, Koya D, Uzu T, Kashihwagi A. Asialoerythropoietin prevents contrast-induced nephropathy. J. Am. Soc. Nephrol., 19, 321–328 (2008).

18) Zadori ZS, Toth VE, Feher A, Philipp J, Nemeth J, Gyires K. Evidence for the gastric cytoprotective effect of centrally injected agmatine. Brain Res. Bull., 108, 51–59 (2014).

19) Piletz JE, Aricioglu F, Cheng JT, Fairbanks CA, Gilad VH, Hae-nisch B, Halasir A, Hong S, Lee JE, Li J, Liu P, Molderings GJ, Rodrigues AL, Satriano J, Seong GJ, Wilcox G, Wu N, Gilad GM. Agmatine: clinical applications after 100 years in translation. Drug Discov. Today, 18, 880–893 (2013).

20) Lauver DA, Carey EG, Bergin IL, Lucchesi BR, Gurm HS. Silde-naphil citrate for prophylaxis of nephropathy in an animal model of contrast-induced acute kidney injury. PLOS ONE, 9, e113598 (2014).

21) Andrade L, Campos SH, Seguro AC. Hypercholesterolemia ag-grates radiocontrast nephrotoxicity: protective role of L-arginine. Kidney Int., 53, 1726–1729 (1998).

22) Schwartz D, Blum M, Peer G, Wollman Y, Maree J, Grosskopf I, Cabi, Levo Y, Iainza A. Role of nitric oxide (EDRF) in radiocontrast acute renal failure in rats. Am. J. Physiol., 267, F374–F379 (1994).

23) Gleeson TG, Bulugahapitiya S. Contrast-induced nephropathy. AJR Am. J. Roentgenol., 183, 1673–1689 (2004).