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Investigating the Possible Protective Role of Direct Intra-arterial Administration of Mannitol and *N*-Acetylcysteine and Per Os Administration of Simvastatin Against Contrast-Induced Nephropathy: An Experimental Study in a Rabbit Model

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Abstract

Purpose Contrast-induced nephropathy (CIN) is one of the leading causes of hospital-acquired acute kidney injury due to the use of iodinated contrast media in various interventional procedures like endovascular aneurysm repair. Its pathophysiology remains mostly unclear. The purpose of the present study was to comparatively study the possible protective role of direct intra-arterial administration of mannitol and acetylcysteine and per os administration of simvastatin in a histopathological level.

Materials and Methods In the present study, we administered iopromide directly in the infrarenal aorta of 24 New Zealand white rabbits after laparotomy. Animals were divided in four groups of six: G1 received iopromide with no protection, G2 iopromide with mannitol, G3 iopromide with acetylcysteine, and G4 iopromide with simvastatin. Renal function blood parameters were assessed prior to the administration, and in 48 h; histopathological evaluation of the kidneys was performed.

Results CIN was evident only in the no protection group G1. Moreover, G1 demonstrated significantly more severe lesions than groups G2, G3, and G4 regarding histopathological findings in glomeruli, vacuolization of tubular epithelial cells, tubular proteinaceous casts, and tubular necrosis. According to our results, intra-arterial administration of mannitol seems to be effective in protection against tubular necrosis.

Conclusion In general, all three agents demonstrated a protective role in preventing the development of CIN, although it seems that there are various pathways that remain to be investigated further.

Keywords Contrast-induced nephropathy · Acetylcysteine · Mannitol · Simvastatin · Intra-arterial · Rabbit model · CIN protection

Introduction

According to a large number of researchers, CIN is the third leading cause of hospital-acquired renal impairment, accounting for about 10% of all cases and is associated with a mortality rate ranging from 3.8 to 64% [1, 2]. A wide variety of CIN incidence has been reported between low- and high-risk populations from 2 to 50%, respectively. Common risk factors include previous chronic renal insufficiency, diabetic nephropathy, heart failure, advanced age, anemia, and reduced effective circulating volume [3]. Moreover, the increasing number of complex intra-arterial interventions contributes to the high incidence of CIN, due

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to the use of higher volume of contrast media. The incidence of CIN remains high despite the introduction of newer and safer contrast media and improved hydration protocols. Sufficient hydration (sodium bicarbonate SB and sodium chloride) and careful use of appropriate renoprotective drugs (*N*-acetylcysteine NAC, vitamin C, adenosine antagonists Aas, statins, loop diuretics, and agiotensin-converting enzyme inhibitors ACEIs) are common strategies in the management of this complication. Avoidance of nephrotoxic drugs and minimization of the volume of contrast agent are also important, but most of the other pharmacological interventions against CIN have proven to be largely ineffective in the clinical setting [4, 5] Therefore, the development of novel therapeutic interventions continues to be a topic of intense research interest. Although its pathophysiology is unclear, there are a number of mechanisms relative to direct tubular renal cell toxicity and glomerural and arteriolar injury of the renal cell due to hemodynamic alterations and oxidative stress. Numerous clinical studies tried to explain the pathophysiology of CIN. The major disadvantage of these studies is that they cannot evaluate the injury in histopathological level. That was the reason why a number of experimental contrast-induced nephropathy (CIN) animal models were developed to project the findings to humans [6–10]. The present study aimed to comparatively study the possible protective role of direct intra-arterial administration of mannitol and acetylcysteine and per os administration of simvastatin in a histopathological level.

Materials and Methods

Experimental Protocol

Twenty-four New Zealand white rabbits 3–4 months old, weighing approximately 3 kg, were divided into four groups of six and were housed at standard conditions with access to standard rabbit food and tap water ab libitum.

All four groups have undergone standard laparotomy under general anesthesia. The aorta and both renal arteries were identified and dissected. The infrarenal aorta was cannulated with the use of a small (20–24G) vein catheter, and iopromide solution (ULTRAVIST® 300 INJ.SOL 62.34% (30% iodine), Bayer Healthcare, Berlin, Germany) was administered manually at a dose of 5 g/kg bodyweight (BW) [11]. After the iopromide administration, the wound was surgically closed, and the animals were left to recover. In the first group (G1), we administered iopromide alone. In groups 2, 3, and 4 (G2, G3, G4) before the administration of iopromide, we administered, as prophylactic agents, mannitol (0.2 g/kg BW, 3 min before), acetylcysteine (150 mg/kg BW, 5 min before), and simvastatin (30 mg/kg BW the day before the operation via an orogastric tube), respectively. At 48 h, the animals were euthanized according to the guidelines and regulations for animal experimentation (i.v. Pentobarbital Sodium-Dolethal®, 5 ml per animal). Blood samples were collected before the first administration of the contrast medium and just before the sacrifice of the animals at 48 h.

Renal Function Parameters

Serum creatinine and urea were calculated in blood samples taken at 0 h and 48 h with the use of standard absorbance photometry.

Histopathological Examination of Renal Tissue

The histopathological evaluation (Figs. 1, 2, 3, 4) was performed by two separate pathologists, with the use of a light microscope, blinded to study data. The histopathological lesions regarding the glomeruli were evaluated depending on cellularity in extracellular matrix, the presence of fibrin, sclerosis, or implementation and crescent. Histopathological changes regarding glomeruli, vacuolization of tubular cells, loss of brush border (microvilli) of the proximal convoluted tubule, tubular proteinaceous casts, and tubular necrosis are expressed as the total number of kidneys according to the extent of the lesion [12]. Specifically:

- Glomeruli lesions (0—no injury, 1—injury up to one-third, 2—one-third up to two-thirds injury, 3—injury of more than two-thirds).
- Vacuolization of tubular cells (0—no injury, 1—injury up to 25% of tubules, 2—injury between 25 and 50%, 3—injury of more than 50%).
- Loss of brush border (microvilli) of the proximal convoluted tubule (0—no injury, 1—injury up to 25% of proximal convoluted tubules, 2—injury between 25 and 50%, 3—injury of more than 50%).
- Tubular proteinaceous casts (0—no injury, 1—injury up to 25%, 2—injury between 25 and 50%, 3—injury of more than 50%).
- Tubular necrosis (0—no injury, 1—injury up to 10%, 2—injury between 10 and 25%, 3—injury of 25–50%, 4—injury of 50–75%, 5-injury of more than 75%).

Statistical Analysis

The minimum sample needed for this study was calculated as follows: Each animal was chosen as the experimental unit. It was estimated that a minimum sample size of six rabbits per group (24 in total) has a power of 0.80 to detect

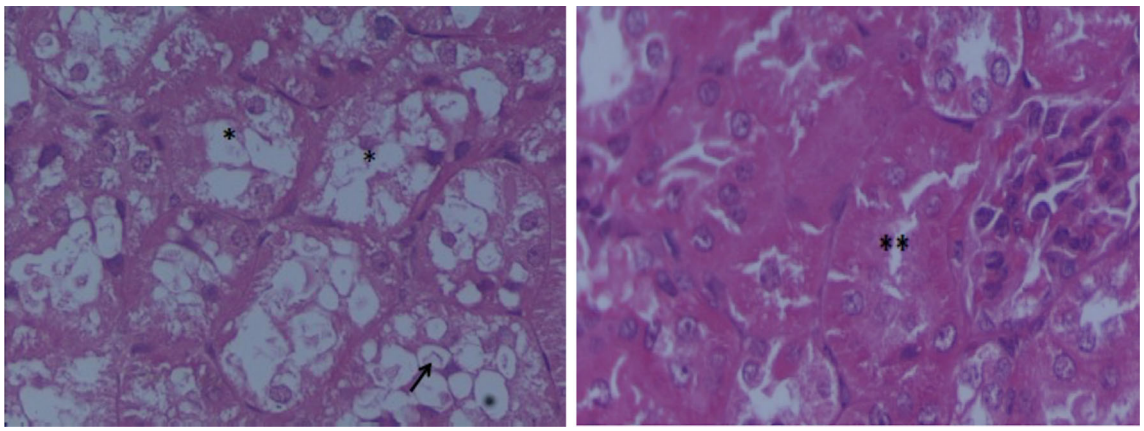

Fig. 1 Vacuolization of tubular epithelial cells (*), shedding of vacuolated cells into lumen (arrow), loss of brush border (**). Histopathological examination preparation consisted of 24-h fixation of both kidneys of each animal in phosphate-buffered formalin solution 10%. The kidneys were sectioned (longitudinally) in a saggital plane and embedded in paraffin blocks. Sections of 4 μm were cut on a rotary microtome, and kidney sections were stained with hematoxylin and eosin (H&E) and Periodic acid–Schiff (PAS), the latter for better visualization of the basic cell membranes and the extracellular matrix

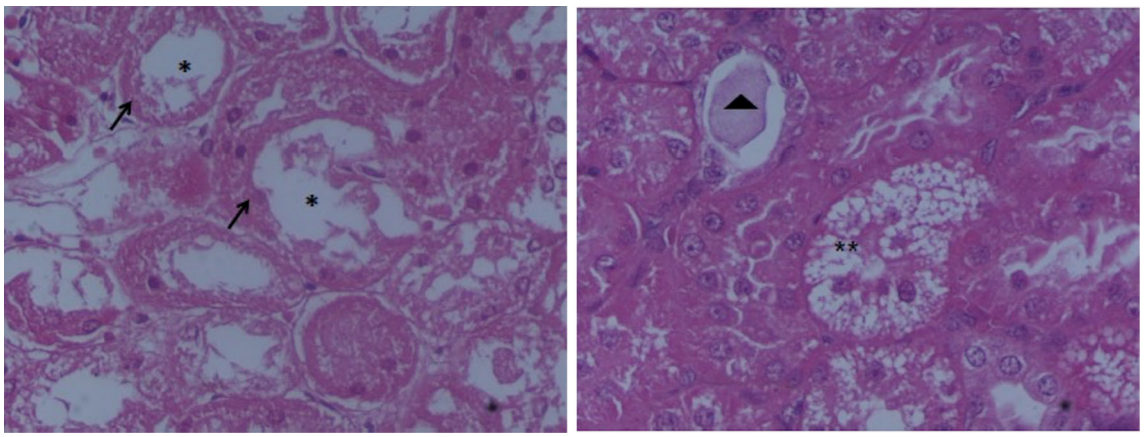

Fig. 2 Tubular necrosis: loss of individual lining cells (loss of nuclei) (arrow), dilatation of the lumen (*asterisk), cytoplasmic basophilia. Vacuolization of tubular epithelial cells (**), hyaline cast (head of arrow)

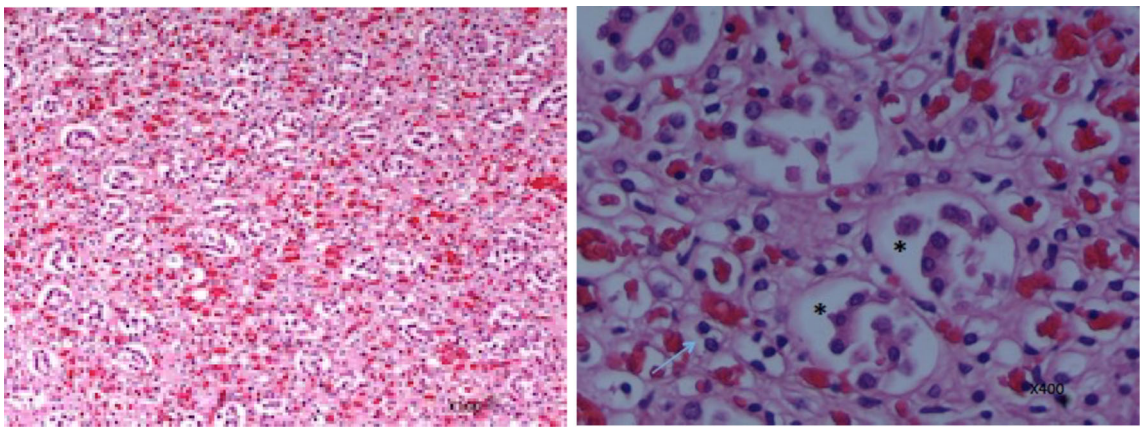

Fig. 3 Tubular necrosis (extensive): severe sloughing of cells into the lumen (*asterisk), cytoplasmic basophilia, separated tubules from one another, inflammatory cells and hemorrhage in the interstitium

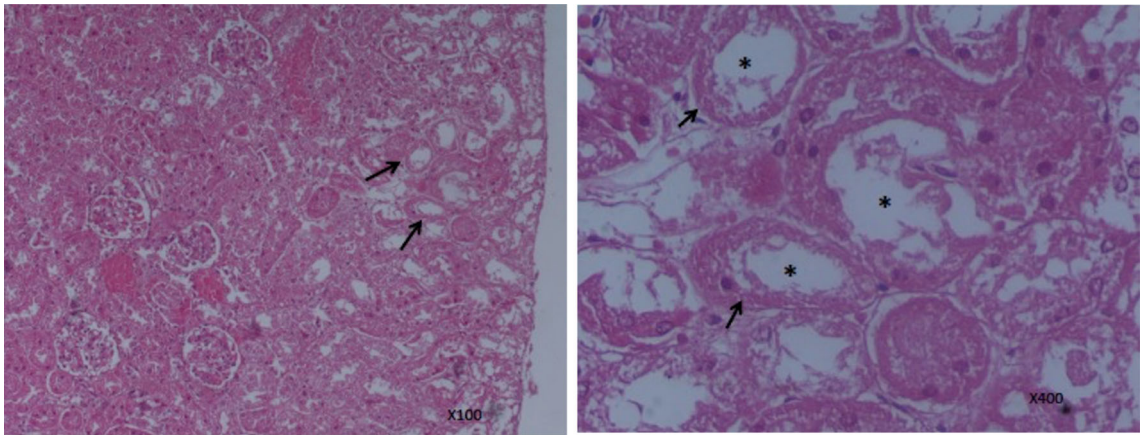

Fig. 4 Tubular necrosis (extensive): loss of individual lining cells (loss of nuclei) (arrow), dilatation of the lumen (*asterisk), cytoplasmic basophilia

an absolute difference (D), between any two groups, of 1 grade (± 0,5 SD within each group) in the histopathological lesion severity, at a significance level $a = 0.05$ (for a two-tailed Mann–Whitney test). This difference corresponds to an effect size $d = 2$. A priori power analysis was accomplished using the G*Power v.3.1.0 software [13]. The difference of 1 grade was considered of clinical importance, and a common SD of 0.5 was estimated according to previous studies [12, 14].

Data were summarized by means of descriptive statistical indices (min, max, median values, and mean values ± standard deviation). Statistical comparisons between groups relative to the distribution of the severity of the lesions were performed with Chi-square test. For statistical comparisons between groups regarding the central tendency of the severity of the lesions, Kruskal–Wallis test and Mann Whitney test were performed. In all hypotheses testing procedures, the observed significance level (p value) was computed by the Monte Carlo simulation method. This approach leads to valid inferences even in cases where the methodological presuppositions of the nonparametric tests are not satisfied [15]. All analyses were performed with SPSS v15.0 statistical package enhanced with the module Exact Tests. The statistical significance level of all were predetermined at $p < 0.05$.

Results

Clinical Evaluation of the Animals

Postoperatively, animals were left at standard conditions with rabbit food and tap water ab libitum. All the animals recovered well from the surgery with no signs of discomfort. They were inspected three times a day, with no signs of anorexia, dehydration, or any other distress.

Histopathological Evaluation of Kidney Tissue

Histopathological Findings in Glomeruli

Group G1 (no protection group) demonstrated more lesions than G2 (mannitol) ($p = 0.026$), G3 (acetylcysteine) ($p = 0.001$), and G4 (simvastatin) ($p < 0.001$). G2 had significantly more severe lesions than G4 ($p = 0.002$) but did not differ from G3. No statistically significant differences were noted between G3 and G4 (Table 1).

Arteriolar Injury

The histopathological examination did not reveal any distinct arteriolar injury.

Tubulointerstitial Injury

Tubular Vacuolization In G1, all the examined kidneys demonstrated lesion severity regarding vacuolization of tubular epithelial cells (Fig. 1) from 1 to 3, while no injury was demonstrated in at least 50% of the kidneys in G2, G3, and G4. Additionally, these latter groups demonstrated no level 3 injuries. G1 demonstrated statistically significant more tubular vacuolization than G2 ($p < 0.001$), G3 ($p = 0.005$), and G4 ($p = 0.028$). Groups G2, G3, and G4 did not differ (Table 2).

Loss of Brush Border (Microvilli) In G1 and G2, all the examined kidneys demonstrated minor lesion severity of level 1 regarding the loss of brush border (microvilli) of the proximal convoluted tubule, while no injury demonstrated the majority of G4. G4 demonstrated statistically significant, less loss of brush border (microvilli) of the proximal convoluted tubule than G1 ($p = 0.001$) G2 ($p = 0.002$) and

Table 1 Histopathological lesions regarding glomeruli

Group		0	1	2	3	Total	Mean	SD	Median
Severity of glomerular lesions (0, 1, 2, 3)									
G1	*n* (%)	0	5	5	2	12	1.75	0.754	2
		0.0%	41.7%	41.7%	16.7%	100.0%			
G2	*n* (%)	2	8	2	0	12	1	0.603	1
		16.7%	66.7%	16.7%	0.0%	100.0%			
G3	*n* (%)	7	4	1	0	12	0.5	0.674	0
		58.3%	33.3%	8.3%	0.0%	100.0%			
G4	*n* (%)	10	2	0	0	12	0.17	0.389	0
		83.3%	16.7%	0.0%	0.0%	100.0%			

There are statistically significant differences between the four groups relative to the distribution of the lesion severity in the glomerular part of the kidneys (*Chi-square* test, $p < 0.001$). In addition, the Kruskal–Wallis test shows that there are also statistically significant differences between the four groups relative to the central tendency of the glomerular lesion severity (Kruskal–Wallis test $p < 0.001$)

$\chi^2 = 30.158$ $df = 9$ $p < 0.001$—Kruskal–Wallis $p < 0.001$

Table 2 Histopathological changes regarding vacuolization of tubular cells

Group		0	1	2	3	Total	Mean	SD	Median
Cellular vacuolization (0, 1, 2, 3)									
G1	*n* (%)	0	6	5	1	12	1.58	0.669	1.5
		0.0%	50.0%	41.7%	8.3%	100.0%			
G2	*n* (%)	6	6	0	0	12	0.5	0.522	0.5
		50.0%	50.0%	0.0%	0.0%	100.0%			
G3	*n* (%)	6	2	2	0	12	0.83	0.937	0.5
		50.0%	16.7%	33.3%	0.0%	100.0%			
G4	*n* (%)	8	0	4	0	12	0.67	0.985	0
		66.7%	0.0%	33.3%	0.0%	100.0%			

There are statistically significant differences between the four groups relative to the distribution of the lesion severity regarding tubular vacuolization (Chi-square test, $p = 0.003$). In addition, the Kruskal–Wallis test shows that there are also statistically significant differences between the four groups relative to the central tendency of the severity regarding tubular vacuolization (Kruskal–Wallis test $p = 0.001$)

$\chi^2 = 22.453$ $df = 9$ $p = 0.003$ Kruskal–Wallis $p = 0.001$

G3 ($p = 0.013$). Groups G1, G2, and G3 did not differ (Table 3).

Tubular Proteinaceous Casts G1 demonstrated more statistically significant tubular proteinaceous casts than G2 ($p = 0.043$), G3 ($p < 0.001$), and G4 ($p < 0.001$). Additionally, G2 differed from G3 ($p = 0.016$) and G4 ($p = 0.013$). G3 and G4 did not differ (Table 4).

Tubular Necrosis In G2, all the examined kidneys demonstrated no injury regarding the tubular necrosis (Figs. 2, 3, 4), while above 90% of G3 and G4 demonstrated lesion severity 0 to 1. G1 demonstrated statistically significant more tubular necrosis than G2 ($p < 0.001$), G3 ($p = 0.029$), and G4 ($p = 0.005$). Additionally, G2 had statistically significant less lesions than G3 ($p = 0.015$). There were no statistically significant differences between G2–G4 and G3–G4, respectively (Table 5).

Renal Function Parameters (Urea–Creatinine Differences)

In G1, all the examined animals demonstrated difference in creatinine prior and after surgery, which was more than 25% of the initial value. According to the definition of the contrast-induced nephropathy (CIN), all the examined animals which did not have any protection demonstrated CIN, while G2, G3, and G4 groups did not. G1 demonstrated statistically significant increase in serum creatinine than G2 ($p = 0.003$), G3 ($p = 0.043$), and G4 ($p = 0.002$). G3 did not differ from G2 and G4, respectively (Table 6).

Table 3 Histopathological changes regarding the loss of brush border (microvilli) of the proximal convoluted tubule

Group		0	1	2	3	Total	Mean	SD	Median
Loss of brush border (microvilli) of the proximal convoluted tubule (0, 1, 2,3)									
G1	*n* (%)	0	12	0	0	12	1.0	0	1
		0.0%	100.0%	0.0%	0.0%	100%			
G2	*n* (%)	0	12	0	0	12	1	0	1
		0%	100%	0.0%	0.0%	100%			
G3	*n* (%)	2	7	3	0	12	1.08	0.669	1
		16.7%	58.3%	25.0%	0.0%	100%			
G4	*n* (%)	8	4	0	0	12	0.33	0.492	0
		66.7%	33.3%	0.0%	0.0%	100%			

There are statistically significant differences between the four groups relative to the distribution of the lesion severity regarding the loss of brush border (microvilli) of the proximal convoluted tubule (Chi-square test, $p < 0.001$). In addition, the Kruskal–Wallis test shows that there are also statistically significant differences between the four groups relative to the central tendency of the severity of the loss of brush border (Kruskal–Wallis test $p = 0.001$)

$\chi^2 = 31.543$ $df = 6$ $p < 0.001$ Kruskal–Wallis $p < 0.001$

Table 4 Histopathological changes regarding the tubular proteinaceous casts

Group		0	1	2	3	Total	Mean	SD	Median
Tubular proteinaceous casts (0, 1, 2, 3)									
G1	*n* (%)	2	3	6	1	12	1.5	0.905	2
		16.7%	25.0%	50%	8.3%	100.0%			
G2	*n* (%)	6	4	2	0	12	0.67	0.778	0.5
		50.0%	33.3%	16.7%	0.0%	100.0%			
G3	*n* (%)	12	0	0	0	12	0	0	0
		100%	0.0%	0.0%	0.0%	100.0%			
G4	*n* (%)	12	0	0	0	12	0	0	0
		100%	0.0%	0.0%	0.0%	100.0%			

There are statistically significant differences between the four groups relative to the distribution of the lesion severity regarding the tubular proteinaceous casts (Chi-square test, $p < 0.001$). In addition, the Kruskal–Wallis test shows that there are also statistically significant differences between the four groups relative to the central tendency of the severity of the formation of the tubular proteinaceous casts (Kruskal–Wallis test $p < 0.001$)

$\chi^2 = 31.286$ $df = 9$ $p < 0.001$ Kruskal–Wallis $p < 0.001$

Table 5 Histopathological changes regarding the tubular necrosis

Group		0	1	2	3	Total	Mean	SD	Median
Tubular necrosis (0, 1, 2, 3, 4, 5)									
G1	*n* (%)	1	9	0	2	12	1.25	0.866	1
		8.3%	75.0%	0%	16.7%	100%			
G2	*n* (%)	12	0	0	0	12	0	0	0
		100%	0.0%	0.0%	0.0%	100%			
G3	*n* (%)	6	5	1	0	12	0.58	0.669	0.5
		50%	41.7%	8.3%	0.0%	100%			
G4	*n* (%)	8	4	0	0	12	0.33	0.492	0
		66.7%	33.3%	0.0%	0.0%	100%			

There are statistically significant differences between the four groups relative to the distribution of the lesion severity regarding the tubular necrosis (Chi-square test, $p < 0.001$). In addition, the Kruskal–Wallis test shows that there are also statistically significant differences between the four groups relative to the central tendency of the severity of the tubular necrosis (Kruskal–Wallis test $p < 0.001$)

$\chi^2 = 27.407$ $df = 9$ $p < 0.001$ Kruskal–Wallis $p < 0.001$

Table 6 Renal function changes regarding to urea and creatinine

Group		Dif_U	Dif_C
1	Minimum	69.23	32.69
	Median	112.90	46.99
	Maximum	206.67	75.93
	Mean	132.40	51.07
	Std. error of mean	15.40	4.21
	SD	53.33	14.59
2	Minimum	− 14.29	0.00
	Median	8.82	3.61
	Maximum	33.33	18.75
	Mean	8.26	6.71
	Std. error of mean	5.03	2.19
	SD	17.42	7.57
3	Minimum	0.00	− 1.96
	Median	25.00	12.50
	Maximum	400.00	75.00
	Mean	101.39	20.28
	Std. error of mean	42.65	7.54
	SD	147.75	26.13
4	Minimum	14.29	12.50
	Median	36.67	16.88
	Maximum	66.67	28.57
	Mean	35.35	18.86
	Std. error of mean	5.27	1.82
	SD	18.26	6.29
Total	Minimum	− 14.29	− 1.96
	Median	33.33	14.64
	Maximum	400.00	75.93
	Mean	69.35	24.23
	Std. error of mean	13.27	3.25
	SD	91.92	22.49

Data are expressed as the difference in serum urea and creatinine prior and after surgery (48 h) in mg/dl. The Kruskal–Wallis test shows that there are statistically significant differences between the four groups relative to the value changes of serum urea ($p = 0.002$) and serum creatinine ($p = 0.002$)

Kruskal–Wallis Dif U $p = 0.002$

Kruskal–Wallis Dif Cr $p = 0.002$

Discussion

According to Linkermann et al. [16] and Tsamouri et al. [12], the perfect rabbit CIN model should consist of three parameters: The i.v. and contrast medium administration should significantly increase the serum creatinine levels, the histopathological changes should be easily diagnosed with common stains, and the model should not be lethal. In fact, all existing animal models are imperfect considering the 'normal' renal function of the animals prior to the exposure to the pathogenic agent and CIN induction. The majority of these animal studies utilize rats despite the difficulties in CIN induction [5]. There is a large variety of protocols used involving the induction of prerenal azotemia (16–24-h water deprivation), partial kidney resection accompanied by 48-hour water deprivation, or preceded nephrotoxic drug administration prior to contrast medium administration [6–9]. In our experimental model, we used New Zealand white rabbits for a number of reasons. At first, it is an increasingly used model [14, 17], and despite the rabbit's resemblance to rodents, protein sequence data suggest that rabbits are more closely related to primates with a concurrent sensitivity to contrast medium more prevalent than that in rats [11, 18]. Moreover, New Zealand white rabbits appear to be more vulnerable to the administration of CM and thus more susceptible to CIN than other strains of rabbits. In our experimental model, CIN was induced in healthy rabbits with a single intra-aortic suprarenal injection of CM and clamping of the infrarenal aorta, after laparotomy under general anesthesia, considering all the above as a reliable nephrotoxicity rabbit model [9]. All animals of the present study were healthy with normal renal function in contrast with previous studies [6, 8].

Serum creatinine levels should increase 25% or more in relation to the baseline values in order to come under the definition of CIN. Lauver et al. [14] showed that creatinine begun to rise in 1–2 h and peaked at 48 h post-contrast media administration. Tsamouri et al. [12] showed that serum biochemical parameters showed an increase in serum creatinine levels above 25% at 2 h, immediately after contrast administration, which is typical for renal function impairment according to the European Society of Urogenital Radiology [19]. In our model, all G1 animals demonstrated CIN and persistent high levels of serum creatinine 48 h after contrast medium administration. On the contrary, all three other groups did not demonstrate CIN. The mannitol group showed a relative statistically significant smaller increase in serum creatinine than the group of simvastatin which indicates a relative protective role of mannitol that should be studied further. In general, all three agents demonstrated a protective role in preventing the development of CIN.

The present study comparatively examined the possible protective role of the intra-arterial administration of mannitol and acetylcysteine in relation both to the biochemical parameters and the glomerural, the arterioral, and the tubular histopathological findings. We also examined the role of the per os administration of simvastatin looking for hard histopathological evidence. The histopathological findings were associated well with the biochemical markers of CIN. According to our analysis, the "no-protection" group demonstrated more glomerular lesions than any

other group of the study, while the mannitol and acetylkysteine group had significantly more severe lesions than simvastatin group, which is indicative of the protective role of the given substances and especially of the protective role of statin. The histopathological examination did not reveal any distinct arteriolar injury.

Vacuolization of tubular epithelial cells is a degenerative change associated with a variety of toxic substances. CM is taken up by tubular cells by pinocytosis and pinocytic vesicles fuse with lysosomes forming larger vacuoles [12, 20]. Usually, cells are "swollen" and nuclei are dislocated. Although tubular vacuolization does not directly correlate with renal function impairment, as it does not cause the loss of renal function itself and resolves spontaneously, it is a consequence of pinocytosis and lysosomal function, which, although not specific, is the earliest marker of contrast-induced nephropathy [9]. In our study, the "no-protection" group showed statistically more tubular vacuolization than all the other groups. The two-third of the simvastatin group demonstrated no vacuolization of tubular epithelial cells.

One of the more indicative markers of direct cell toxicity of the tubular epithelial cells is the loss of the microvilli border. Practically, no microvilli loss was present in the G4, indicating a possible protective role of simvastatin. This nephroprotective effect could possibly be due to antioxidant, anti-inflammatory, antithrombotic, and vasodilator properties mediated by NO that improve renal microcirculation [21].

Proteinaceous casts represent fluid accumulation or cell breakdown products that fill the tubular lamina [12, 22–24]. Tubular obstruction by proteins is associated with CIN. All three agents seem to play a protective role, minimizing the accumulation of proteinaceous casts in the tubular lumen.

The most important histopathological lesion related to CM acute nephrotoxicity is necrosis, especially in proximal convoluted tubules. Our results are in line with previous research in rats [24, 25] and rabbits [12, 14] given the fact that proximal convoluted tubules are more susceptible to necrosis than medullary collecting ducts. Generally, the tubular part of the kidney is where the most active transport activity occurs with increased ion permeability and chemical influx. The goal of prevention is to protect the renal tubules from prolonged contact with contrast material, because permanent damage can occur at the time of contact. According to our results, mannitol group (G2) showed no signs of tubular necrosis. Additionally, mannitol seems to be more protective regarding tubular necrosis than acetylcysteine, but there seems to be no other statistically significant differences between the other groups (mannitol–simvastatin or acetylcysteine–simvastatin). The role of mannitol in tubular necrosis protection has been controversial in previous studies [26]. Probably, intra-arterial administration decreases the contact time of contrast agent within the kidney, thus decreasing nephrotoxic effects on tubular epithelium [27]. The value of intra-arterial use of known compounds with diuretic effect remains controversial, warranting further clinical investigation.

Limitations of the Study

Animal model experimental studies imply deviation from reality usually by simplifying and reducing variables. Moreover, the selected group sizes have been the smallest possible, so a significant result can be expected according to a priori power analysis.

Conclusion

Overall, all three protective agents, simvastatin, acetylcysteine, and mannitol, appeared efficient in preventing CIN in our rabbit model according to the definition, which considers the 25% increase in serum creatine as a prerequisite. Additionally, in regard to the histopathological findings, all three agents seem to play a significant protective role with minimal lesions of glomeruli, tubular epithelial cells, microvilli border, and necrosis with no findings of proteinaceous casts. Intra-arterial administration of mannitol seems to be effective in protection against tubular necrosis. All agents exhibit protective properties against CIN, and it seems that there are various pathways that remain to be investigated further.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This experimental study strictly met all the criteria and the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institute of Health. The protocol was approved by the National Committee of Agricultural Economy and Veterinary Medicine of Central Macedonia (Thessaloniki, 29 September 2015, Αριθμ. Πρωτ. 326491/2358) according to the 2010/63/EU directive of the European Parliament and of the Council (2 September 2010, on the protection of animals used for scientific purposes) and the relevant adaptation of the Greek law (L 276/33/20.10.2010, ΦΕΚ 106/τ.Α/30.04.2013). All operations were performed in the veterinary clinic of Aristotle University of Thessaloniki under general anesthesia, with the use of ketamine and/or sodium pentobarbital, induced by a veterinary anesthesiologist and all efforts were made to minimize suffering.

Human and Animal Rights This article does not contain any studies with human participants performed by any of the authors.

Informed Consent For this type of study, informed consent is not required.

Consent for Publication For this type of study, consent for publication is not required.

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