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Effect of the Direct Renin Inhibitor Aliskiren in the Prevention of Experimental Contrast-Induced Nephropathy in the Rat

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Key Words
Aliskiren · Contrast-induced nephropathy · Acute renal failure · Experimental study

Abstract
Background: Renal vasoconstriction, activated by the renin-angiotensin system, plays a pivotal role in the pathogenesis of contrast-induced nephropathy (CIN). The purpose of this study was to evaluate the effect of aliskiren, a direct renin inhibitor, for the prophylaxis of experimental CIN in the rat. ***Methods:*** Thirty-two Wistar albino rats were divided into four groups of 8 rats each, namely the control (C), aliskiren (A), contrast media (CM) and aliskiren plus contrast media (ACM) groups. Aliskiren was given orally at a dose of 50 mg/kg/day once daily for 5 consecutive days. CIN was induced by intravenous administration of indomethacin, N-nitro-L-arginine methyl ester and high-osmolar contrast medium meglumine amidotrizoate. Renal function parameters, kidney histology and tubular expression of vascular endothelial growth factor were determined. ***Results:*** Mean serum creatinine was significantly lower ($p < 0.001$) and mean creatinine clearance was higher ($p < 0.001$) in the ACM group compared with the CM group. However, there were no differences between the ACM and CM groups in terms of tubular necrosis, proteinaceous casts, medullary congestion and vascular endothelial growth factor expression. ***Conclusion:*** Our preliminary data seem to suggest a potential role of aliskiren for the prophylaxis of CIN in an experimental rat model.

Introduction

Contrast-induced nephropathy (CIN), defined as the rapid deterioration in renal function after administration of contrast medium (CM) without other specific causes, is a common cause of acute kidney injury [1–3]. Patients who develop CIN are at a higher risk of morbidity and mortality compared with controls [4, 5]. For these reasons, precautionary maneuvers are warranted to reduce the incidence of this complication, especially in subjects with an underlying renal insufficiency [6–8]. Unfortunately, CIN remains poorly understood despite decades of investigation, and effective means of reducing its incidence are still lacking [9].

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Previous data have shown that the renin-angiotensin-aldosterone system (RAAS) may play a role in the pathophysiology of CIN [10]. However, there is still controversy concerning the clinical usefulness of RAAS blockade in the prevention of this condition [11]. Some studies have demonstrated that chronic medication with angiotensin-converting enzyme inhibitors (ACE-I) or angiotensin II receptor antagonists may represent a risk factor for CIN [12–14]. In contrast, other authors have reported a protective effect of ACE-I in patients with chronic kidney disease exposed to CM [10].

Aliskiren – 2(S),4(S),5(S),7(S)-N-(2-carbamoyl-2-methylpropyl)-5-amino-4-hydroxy-2,7-diisopropyl-8-[4-methoxy-3-(3-methoxypropoxy)phenyl]-octanamide hemifumarate – is a direct renin inhibitor that has been approved for lowering arterial blood pressure [15, 16]. The drug occupies a specific site within the enzymatic pocket of the renin/prorenin receptor, thereby blocking the function of this enzyme [17]. Because the RAAS cascade begins with renin and because aliskiren may exert renoprotective effects [18–20], we aimed to examine the effects of aliskiren for prevention of CIN using a rat model. In addition – as aliskiren may reduce vascular endothelial growth factor (VEGF) expression [21], which is increased in acute kidney injury [22] – we assessed whether tubular VEGF expression can be diminished by the administration of aliskiren immediately before the experimental induction of CIN. The potential usefulness of direct renin inhibition for preventing CIN has not been previously examined neither in animal models nor in humans. This strategy is particularly interesting as a complete blockade with the use of ACE-I and angiotensin receptor blockers with or without aldosterone receptor blockers is not easy to achieve, and side effects are not uncommon [18].

Methods

Ethics

All in vivo experiments were reviewed and approved by the Committee of Ethics on Animal Experiments, Marmara University School of Medicine, Istanbul, Turkey, according to the Guidelines of the American Physiological Society. This study also conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Animals

Healthy male albino rats of Wistar strain weighing 240–270 g (7 weeks old) bred locally in the central animal house were selected for the study and randomly assigned to four groups of 8 animals each. They were housed in standard stainless steel hanging cages in a vivarium with controlled temperature (22–25°C), humidity (35–50%), and photocycle (12 h light/12 h dark). The animals were placed in metabolic cages to record water intake and 24-hour urine collection.

Experimental Design

The animals (n = 32) were allocated randomly into four equal groups of 8 rats each. Animals in the control group (C) received saline injections into the tail vein 3 times at 15-min intervals. Rats in the aliskiren (A) group received aliskiren (Novartis Pharmaceutical, New York, N.Y., USA) 50 mg/kg/day by oral gavage once daily for 5 consecutive days before the experimental induction of CIN. Rats in the CM group underwent experimental induction of CIN by intravenous administration of indomethacin (10 mg/kg), N-nitro-L-arginine methyl ester (10 mg/kg, twice at 15 and 30 min) and high-osmolar contrast medium meglumine amidotrizoate 60% (6 ml/kg; Urovist-Angiographin, Schering AG, Germany), as previously described [23, 24]. Rats in the aliskiren plus CM (ACM) group received aliskiren 50 mg/kg/day by oral gavage once daily for 5 consecutive days before the experimental induction of CIN. All rats were then allowed to recover for 24 h in metabolic cages. Twenty-four-hour urine samples were collected for the assessment of creatinine clearance (CrCl) and fractional excretion of sodium. Blood samples were obtained from the abdominal aorta under general anesthesia after intraperitoneal injection of ketamine (50 mg/kg Ketalar, Pfizer, Istanbul, Turkey). Serum was separated and aliquots were stored at –80°C until analysis.

Renal Function Parameters

Creatinine measurements in serum and urine were performed using a standard spectrophotometric assay (Modular P, Roche Diagnostics GmbH, Penzberg, Germany). Sodium concentrations in serum and urine were measured by ion-selective electrodes (Modular ISE, Roche Diagnostics). CrCl was calculated according to the formula: UV/P, where U represents the urinary creatinine concentration (mg/dl), V is the urinary collection volume (ml/min/100), and P is the serum creatinine concentration (mg/dl). CrCl values were expressed as ml/min/100 g body weight. Fractional excretion of sodium was calculated as follows: (urine sodium/serum sodium) × (serum creatinine/urine creatinine) × 100.

Histology

Kidneys were removed from mice killed by sodium pentobarbital injection (100 mg/kg intraperitoneally), placed in fixative for 4 h at 4°C and then transferred to 2% paraformaldehyde at 4°C until paraffin embedding, sectioning and hematoxylin and eosin staining. Tubular necrosis, proteinaceous casts and medullary congestion were analyzed semiquantitatively according to previous methodology [23]. Tubular necrosis and proteinaceous casts were graded as follows: 0 = no damage; 1 = mild (unicellular, patchy isolated damage); 2 = moderate (damage <25%); 3 = severe (damage between 25 and 50%), and 4 = very severe (>50% damage). The degree of medullary congestion was defined as: 0 = no congestion; 1 = mild (vascular congestion with identification of erythrocytes by ×400 magnification); 2 = moderate (vascular congestion with identification of erythrocytes by ×200 magnification); 3 = severe (vascular congestion with identification of erythrocytes by ×100 magnification), and 4 = very severe (vascular congestion with identification of erythrocytes by ×40 magnification) [23]. Evaluations were made in a blinded manner.

Table 1. Renal function parameters in the four treatment groups (n = 8 rats each)

	C	A	CM	ACM
Serum creatinine, mg/dl	0.37 ± 0.54	0.45 ± 0.08	1.01 ± 0.43	0.52 ± 0.12*
Creatinine clearance, ml/min	1.92 ± 0.67	1.42 ± 0.50	0.57 ± 0.37	0.90 ± 0.37*
Fractional excretion of sodium, %	0.16 ± 0.05	0.19 ± 0.51	1.8 ± 2.19	0.23 ± 0.07*

Values are expressed as means ± standard deviations. * $p < 0.001$ versus the CM group.

Table 2. Histology findings in the four treatment groups (n = 8 rats each)

	C	A	CM	ACM
Tubular necrosis	0.25 ± 0.46	0.28 ± 0.48	2.50 ± 0.53	2.75 ± 0.46
Proteinaceous casts	0.37 ± 0.51	0.14 ± 0.37	2.87 ± 0.64	3.00 ± 0.53
Medullary congestion	0.75 ± 0.88	0.85 ± 0.37	3.50 ± 0.53	3.00 ± 0.53
VEGF expression	0.50 ± 0.53	0.42 ± 0.53	2.62 ± 0.51	2.37 ± 0.51

Values are expressed as means ± standard deviations. No significant differences were found in the CM and ACM groups.

Immunohistochemistry for VEGF

Immunohistochemistry for VEGF in the tubular cells of the kidney outer medulla was performed on 3-μm-thick renal sections using an avidin-biotin-peroxidase technique. Briefly, paraffin-embedded tissue sections were cleared in xylene and rehydrated in a series of ethanol washes. Endogenous peroxidase activity was inhibited by using 3% hydrogen peroxide. Antigen retrieval was performed by microwaving sections in citrate buffer (pH 6). Sections were blocked in phosphate-buffered saline (pH 7.4) for 20 min at room temperature. Protein blockage was performed to reduce nonspecific background staining. The sections were incubated for 30 min with a primary anti-VEGF antibody (Ab-1, RB-222-R7; Thermo Fisher Scientific, Fremont, Calif., USA) and subsequently exposed for 10 min to a biotin-conjugated secondary antibody (UltraVision Detection System; TP-015-HD; Thermo Fisher Scientific). This biotinylated secondary antibody was visualized after reaction with streptavidin-peroxidase conjugate and subsequent addition of diaminobenzidine. Negative controls were performed by omitting the primary antibody. According to previous methodology [23], the percentage of VEGF-positive tubular cells was semiquantitatively assessed by two independent observers and scored as: 0 = negative, no expression of VEGF; 1 = weak staining, 0–25% of tubular cells show positive staining; 2 = moderate staining, >25–75% of tubular cells with positive staining, and 3 = strong staining, >75–100% of tubular cells [23].

Data Analysis

The statistical package SPSS 14.0 (SPSS Inc., Chicago, Ill., USA) was used for data analysis. Shapiro-Wilk tests were performed to check for normal distribution. According to these tests, continuous data were normally distributed and only parametric analyses were exploited. Group comparisons were performed using one-way ANOVA followed by Tukey's pairwise multiple comparison test. Data are presented as means ± standard deviations, and values of $p < 0.05$ (two-tailed) were considered statistically significant. As this study was essentially exploratory in nature, a Bonferroni adjustment was not performed.

Results

The baseline characteristics were well balanced among the four treatment groups. All animals survived to study completion.

Renal Function Parameters

Table 1 shows the renal function parameters in the four treatment groups. Serum creatinine did not differ in the C and A groups. As expected, creatinine levels increased significantly in the CM group compared with control animals ($p < 0.001$). However, there were no significant differences in creatinine values in the ACM group compared with controls. The mean CrCl did not differ in the C and A groups. CrCl was significantly lower in the CM than in the C group ($p < 0.001$), but no differences were seen in the ACM group compared with control rats. The fractional excretion of sodium was sim-

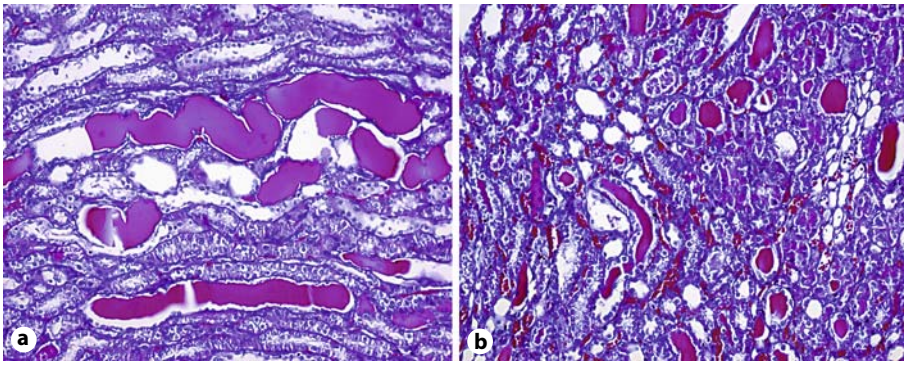

Fig. 1. Presence of areas of tubular necrosis in the outer medulla of CM rats (hematoxylin and eosin; **a**) and ACM rats (**b**). ×200.

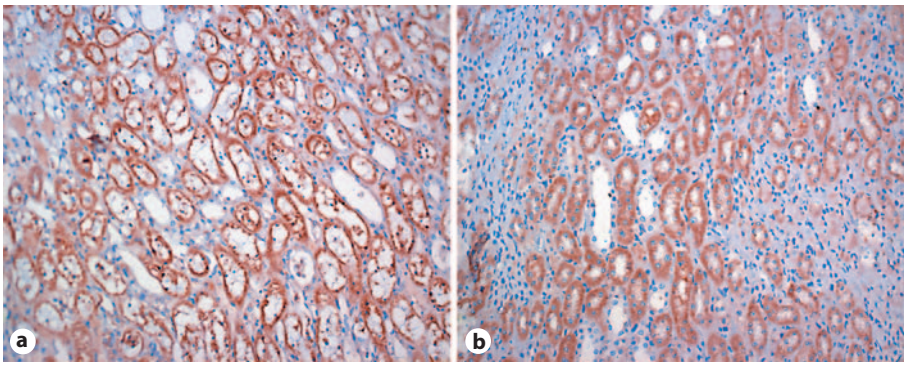

Fig. 2. Intense expression of VEGF in the outer medulla of CM rats (**a**) and ACM rats (**b**). ×200.

ilar in the C and A groups. The mean fractional excretion of sodium increased significantly in the CM group compared with the C group ($p < 0.001$). On the other hand, this parameter was significantly lower in the ACM than in the CM group ($p < 0.001$).

Histology

The histological findings in the four treatment groups are reported in table 2. There were no significant differences in the mean score for the degree of tubular necrosis in the four study groups. Similarly, we found no differences in the mean scores of proteinaceous casts and medullary congestion (fig. 1).

Immunohistochemistry for VEGF

As expected, the mean immunohistochemical score for VEGF was significantly higher in the CM group than in the control group ($p < 0.001$; table 2). However, the VEGF expression was similar in the ACM and CM groups (fig. 2).

Discussion

In the present experimental study, we have shown for the first time that CIN prophylaxis using the direct angiotensin inhibitor aliskiren is effective in attenuating the acute deterioration in renal function in the rat. However, aliskiren did not reduce tubular necrosis, proteinaceous casts and medullary congestion, as well as VEGF immunoexpression associated with the development of CIN.

CIN ranks third in leading causes of hospital-acquired renal failure, and this condition has been associated with increased costs of medical care during hospitalization and prolongation of hospital stay [25]. Although multiple pharmacological prevention strategies – including the use of dopamine, fenoldopam, atrial natriuretic peptide, mannitol, postprocedural diuretics, endothelin receptor antagonists, calcium channel blockers, prostaglandin E_1, aminophylline or theophylline, statins, and ascorbic acid – have been proposed, results have been mixed [26].

The two major theories of the pathogenesis of CIN are renal vasoconstriction and direct cytotoxic effects of contrast agents [3, 25]. In human subjects, renal blood flow

has been reported to decrease by 30–45% within 2–4 h after the injection of CM, which may lead to deterioration in renal functions because of medullary hypoxia [27, 28]. Another possible cause is the direct cytotoxic effect of the radiocontrast agent, which can cause renal tubular injury [29]. The results of the present study seem to suggest that pretreatment with aliskirin before the injection of a CM may have benefits in terms of serum creatinine, CrCl and fractional excretion of sodium. However, histological parameters did not improve significantly. Based on these findings, we speculate that aliskiren could have exerted its beneficial effects in the prevention of CIN mainly through prerenal mechanisms. Accordingly, direct renin inhibition has been shown to increase renal blood flow to a significantly greater degree than angiotensin-converting enzyme inhibition [30]. The increase in renal blood flow induced by aliskiren may be a response to angiotensin AT1 receptor-dependent reduction in vascular tone in the efferent arteriole. Reduced vascular tone in the efferent glomerular arteriole could be responsible for the decrease in intraglomerular pressure [30]. Gross et al. [19] have recently suggested that aliskiren may be renoprotective regardless of its effect on the vascular tone. Indeed, aliskiren has been shown to have antifibrotic and antiproteinuric effects in a nonhypertensive mouse model for progressive renal fibrosis [19]. Another important feature of aliskiren is its partitioning in the renal tissue, where it localizes in the glomeruli and in the renal cortical arteries at concentrations several fold higher than in plasma [31]. While this compound seems to be useful to prevent the histological effects of chronic renal injury, our report seems to suggest that aliskiren pretreatment does not improve kidney histology after administration of CM. Taken together, our findings suggest that the observed improvements in renal function parameters after the induction of CIN are not mirrored by significant histological changes. Therefore, it is likely that the observed effects of direct angiotensin inhibition in the prophylaxis of CIN could mainly be exerted through prerenal mechanisms.

Several caveats of this report merit comment. The small sample size is an obvious limitation. Although this is an experimental study of CIN prevention in the rat, our report provides a proof of concept and could have implications for further clinical research in humans. Dosage regimens for human use should be further evaluated as the protective effect in clinical practice should be dose dependent [30]. Another important limitation is that we did not attempt to measure proteinuria in the rats.

These limitations notwithstanding, we demonstrate for the first time that administration of aliskiren improves renal function parameters in a rat model of CIN but did not ameliorate tubular necrosis, proteinaceous casts and medullary congestion in the kidney after the administration of contrast. While aliskiren may have a role in the prophylaxis of CIN, further animal and clinical studies are needed before any definite conclusion can be drawn. The observed benefits of aliskiren administration can be associated with an improvement in renal blood flow.

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Disclosure Statement

No conflicts of interest declared.

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