Impact of a High Loading Dose of Atorvastatin on Contrast-Induced Acute Kidney Injury

Cristina Quintavalle, PhD*; Danilo Fiore, PhD*; Francesca De Micco, PhD; Gabriella Visconti, MD; Amelia Focaccio, MD; Bruno Golia, MD; Bruno Ricciardelli, MD; Elvira Donnarumma, PhD; Antonio Bianco, PhD; Maria Assunta Zabatta, PhD; Giancarlo Troncone, MD, PhD; Antonio Colombo, MD; Carlo Brigugli, MD, PhD; Gerolama Condorelli, MD, PhD

Background—The role of statins in the prevention of contrast-induced acute kidney injury (CIAKI) is controversial. Methods and Results—First, we investigated the in vivo effects of atorvastatin on CIAKI. Patients with chronic kidney disease enrolled in the Novel Approaches for Preventing or Limiting Events (NAPLES) II trial were randomly assigned to (1) the atorvastatin group (80 mg within 24 hours before contrast media [CM] exposure; n=202) or (2) the control group (n=208). All patients received a high dose of N-acetylcysteine and sodium bicarbonate solution. Second, we investigated the in vitro effects of atorvastatin pretreatment on CM-mediated modifications of intracellular pathways leading to apoptosis or survival in renal tubular cells. CIAKI (ie, an increase >10% of serum cystatin C concentration within 24 hours after CM exposure) occurred in 9 of 202 patients in the atorvastatin group (4.5%) and in 37 of 208 patients in the control group (17.8%) (P=0.005; odds ratio=0.22; 95% confidence interval, 0.07–0.69). CIAKI rate was lower in the atorvastatin group in both diabetics and nondiabetics and in patients with moderate chronic kidney disease (estimated glomerular filtration rate, 31–60 mL/min per 1.73 m²). In the in vitro model, pretreatment with atorvastatin (1) prevented CM-induced renal cell apoptosis by reducing stress kinases activation and (2) restored the survival signals (mediated by Akt and ERK pathways).

Conclusions—A single high loading dose of atorvastatin administered within 24 hours before CM exposure is effective in reducing the rate of CIAKI. This beneficial effect is observed only in patients at low to medium risk. (Circulation. 2012;126:3008-3016.)

Key Words: apoptosis ■ contrast media ■ kidney ■ prevention ■ statins

Contrast-Induced Acute Kidney Injury

Oxidized contrast media (CM) are used in both diagnostic and interventional cardiovascular procedures. In addition to the risk of allergic reactions, the major concern in regard to CM use is a deterioration of kidney function termed contrast-induced acute kidney injury (CIAKI). The reported incidence of CIAKI varies widely (<1% to >50%), depending on the patient population, the baseline risk factors, and the definition.1 Hemodynamic changes of renal blood flow, which lead to hypoxia of the renal medulla, and direct toxic effects of CM on renal cells are thought to contribute to the pathogenesis of CIAKI.2 We have observed previously both in vitro and in vivo that CM induce apoptotic cell death via 3 important signaling pathways: (1) the reactive oxygen species (ROS) pathway, (2) the Jun N-terminal kinase (JNK)/p38 pathway, and (3) the intrinsic apoptosis pathway, which are triggered by CM in this sequence.3,4 The causal relationship between these 3 sequential pathways supports the investigation of novel therapeutic approaches to prevent CIAKI.

Clinical Perspective on p 3016

Statins exert several effects through their non–lipid-related mechanisms. These so-called pleiotropic effects encompass several mechanisms that modify inflammation responses, endothelial function, plaque stability and thrombus formation, and the apoptotic pathway.5–7 The effectiveness of statin pretreatment in reducing the incidence of CIAKI has been examined in some observational8–10 and randomized studies.11–13 Because of the controversial results, there is a general

Received March 6, 2012; accepted October 22, 2012.

From the Department of Cellular and Molecular Biology and Pathology, Federico II University of Naples, and IEOS, CNR Naples (C.Q., D.F., G.C.); Laboratory of Interventional Cardiology and Department of Cardiology, Clinica Mediterranea, Naples (F.D.M., G.V., A.F., B.G., B.R., C.B.); Fondazione IRCCS-SDN, Naples (E.D.); and Dipartimento di Scienze Biomorfoloziche e Funzionali, Università di Napoli Federico II, Naples (A.B., M.A.Z., G.T.); and Laboratory of Interventional Cardiology, San Raffaele Hospital, Milan (A.C.), Italy.

*The first 2 authors contributed equally to this work.

The online-only Data Supplement is available with this article at http://circ.ahajournals.org/lookup/suppl/doi:10.1161/CIRCULATIONAHA.112.103317/-/DC1.

Correspondence to Gerolama Condorelli, MD, PhD, Department of Cellular and Molecular Biology and Pathology, and IEOS, CNR, Federico II University of Naples, Via Pansini, 5, I-80121, Naples, Italy. E-mail gecondor@unina.it; or Carlo Brigugli, MD, PhD, Laboratory of Interventional Cardiology, Clinica Mediterranea, Via Orazio, 2, I-80121, Naples, Italy. E-mail carlobrigugli@clinicamediterranea.it

© 2012 American Heart Association, Inc.

Circulation is available at http://circ.ahajournals.org

DOI: 10.1161/CIRCULATIONAHA.112.103317
consensus that statins merit further study for the prevention of CIAKI. In the present study, we investigated (1) the in vivo effects of atorvastatin pretreatment on CIAKI and (2) the in vitro effects of atorvastatin pretreatment on CM-mediated modifications of intracellular pathways leading to apoptosis or survival in renal tubular cells.

Methods

Patient Population

The patients included in the present study represent the subgroup with chronic kidney disease (CKD) enrolled in the Novel Approaches for Preventing or Limiting Events (NAPLES) II trial7 (Figure 1). The design of the NAPLES II trial has been reported previously. From January 2005 to December 2008, 1348 naive patients (ie, those not taking statins) scheduled for elective coronary angiography or percutaneous coronary intervention in de novo lesions in native coronary arteries were considered eligible for the study. During the procedure, all eligible patients were randomly assigned to atorvastatin treatment (atorvastatin group) or to no atorvastatin treatment (control group). Randomization was performed by a 1:1 ratio with computer-generated random numbers. Patients randomized in the atorvastatin group started atorvastatin treatment (80 mg) within 24 hours before CM exposure. The prophylaxis for CIAKI in all patients with CKD included (1) N-acetylcysteine (NAC) (Fluimucil, Zambon Group SpA, Milan, Italy; 1200 mg PO twice daily, the day before and the day of administration of CM) and (2) hydration with sodium bicarbonate solution14 (154 mEq/L in dextrose and H2O). It was administered immediately before CM injection, followed by 1 mL/kg per hour for 1 hour with the initial intravenous bolus of 3 mL/kg per hour for 1 hour administration of CM. Additional measurements were performed in all instances in which there was a deterioration of baseline renal function. The primary outcome measure was the development of CIAKI, defined as an increase in sCr concentration above the baseline value at 24 hours after administration of CM.18 Secondary outcome measures were (1) an increase of sCr concentration at 48 hours after administration of CM. Additionally, we defined a decrease in eGFR by 25% at 48 hours from baseline value. To address whether a single high (80 mg) dose of atorvastatin may affect the sCr or sCyC levels, we analyzed an additional 20 patients with CKD not scheduled for CM exposure and not enrolled in the original NAPLES II trial. The clinical characteristics of the 20 enrolled patients are summarized in Table I in the online-only Data Supplement. The trial was conducted in 2 interventional cardiology centers in Italy and was approved by our ethics committees.

Culture Conditions and Reagents

Two cell lines were utilized: Madin Darby distal nonhuman tubular epithelial (MDCK) cells and human embryonic proximal tubules (HK2) cells. MDCK cells were grown in a 5% CO2 atmosphere in Dulbecco’s modified Eagle’s medium containing 10% heat-inactivated fetal bovine serum, 2 mmol/L L-glutamine, and 100 U/mL penicillin-streptomycin. HK2 cell lines were grown in Dulbecco’s modified Eagle’s medium–F12 mixture with 10% heat-inactivated fetal bovine serum, 2 mmol/L L-glutamine, and 100 U/mL penicillin-streptomycin. Cells were routinely passaged when 80% to 85% confluent. Media, sera, and antibiotics for cell culture were from Sigma-Aldrich (Milan, Italy). Protein electrophoresis reagents were from Bio-Rad (Richmond, VA), and Western blotting and enhanced chemiluminescence reagents were from GE Healthcare (Milan, Italy). The following antibodies were used for immunoblotting: anti-β-actin (Sigma-Aldrich), anti-phospho-JNK, anti-caspase-3, anti-phosphoserine15 p53, anti-p53, anti-phospho-Akt, anti-Akt, anti-phospho-ERK, anti-ERK, anti-phospho-JNK, anti-JNK (DB Bioscience, Milan, Italy), and anti-β-actin (Sigma-Aldrich, Milan, Italy). MDCK and HK2 cells were pretreated with atorvastatin at a dose of 0.2 μmol/L19 or 100 μmol of NAC.4 The dose of atorvastatin was selected according to the standard doses used in cell lines. Iodixanol was used in all experiments.

Caspase Assay

The caspase assay was performed with the use of the Colorimetric CaspACE® Assay System (Promega, Madison, WI) according to the manufacturer’s protocol. Briefly, MDCK cells were pretreated with 0.2 μmol/L atorvastatin for 12 hours and then treated for 3 hours with iodixanol. Cells were harvested in caspase assay buffer, and proteins were quantified by Bradford assay. Fifty micrograms of protein was used.

Protein Isolation and Western Blotting

Cellular pellets were washed twice with cold phosphate-buffered saline and resuspended in JS buffer (HEPES 50 mmol/L, NaCl 150 mmol/L, 1% glycercol, 1% Triton X-100, 1.5 mmol/L MgCl2, 5 mmol/L EGTA) containing Proteinase Inhibitor Cocktail (Roche, Basel, Switzerland). Solubilized proteins were incubated for 1 hour on ice. After centrifugation at 13 000 rpm for 10 minutes at 4°C, lysates were collected as supernatants. Eighty micrograms of sample extract was resolved on a 12% sodium dodecyl sulfate–polyacrylamide gel with the use of a mini-gel apparatus (Bio-Rad Laborato-
and transferred to Hybond-C extra nitrocellulose (GE Healthcare). Membrane was blocked for 1 hour with 5% nonfat dry milk in Tris-buffered saline containing 0.05% Tween-20 and incubated overnight at 4°C with specific antibodies. The indicated antibody was used for immunoblotting. Washed filters were then incubated for 45 minutes with horseradish peroxidase–conjugated anti-rabbit or anti-mouse secondary antibodies (GE Healthcare) and visualized with chemiluminescence detection (GE Healthcare).

**Cell Death Quantification**

Cell vitality was evaluated with the CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega), according to the manufacturer’s protocol. The assay is based on reduction of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium inner salt (MTS) to a colored product that is measured spectrophotometrically. Cells were plated in 96-well plates in triplicate, stimulated, and incubated at 37°C in a 5% CO2 incubator. Iodixanol and atorvastatin were used in vitro at doses and times indicated. Metabolically active cells were detected by adding 20 μL of MTS to each well. After 30 minutes of incubation, the plates were analyzed on a Multilabel Counter (Bio-Rad). Apoptosis was also analyzed by propidium iodide incorporation in permeabilized cells by flow cytometry. The cells (2×10^6) were washed in phosphate-buffered saline and resuspended in 200 μL of a solution containing 0.1% sodium citrate, 0.1% Triton X-100, and 50 μg/mL propidium iodide (Sigma-Aldrich). After incubation at 4°C for 30 minutes in the dark, nuclei were analyzed with a Becton Dickinson FACScan flow cytometer. Cellular debris was excluded from analyses by raising the forward scatter threshold, and the DNA content of the nuclei was registered on a logarithmic scale. The percentage of elements in the hypodiploid region was calculated.

**Biological Material**

Exfoliated cell pellets from the urine of 10 enrolled and randomly selected patients (5 in each group) were collected by centrifugation at 1200 rpm for 25 minutes. A fraction of urine samples was sent to the pathologist for cytological analysis and a fraction to the laboratory for in vitro assay. All samples were stored at −80°C for a maximum of 2 months. Urine samples were resuspended in ice-cold TRAP (Tris-HCl 10 mmol/L, pH 7.5, MgCl2 1 mmol/L, EGTA 1 mmol/L, phenylmethylsulfonyl 0.1 mmol/L, β-mercaptoethanol 5 mmol/L, CHAPS 0.5%, and glycerol 10%) and incubated on ice for 1 hour. The lysate was centrifuged for 20 minutes at 13 200 rpm at 4°C. The supernatant was collected. The presence of tubular cells was assessed with the use of morphological criteria on cytospin preparations stained by standard Papanicolaou staining methods. To this end, cell block preparations were employed. To ensure their adequacy, cell blocks were stained with hematoxylin and eosin. Caspase-3 expression was detected with the use of rabbit polyclonal antibody (Cell Signaling 9661, Danvers, MA). Signal was developed by the polyvalent LSAB-peroxidase Dako kit (Dako, Denmark).

**Statistical Analysis**

The sample size was selected to demonstrate a reduction in the primary end point of CIAKI from 20% in the control group to 10% in the atorvastatin group. With the use of a 2-sided χ2 test with a significance level of 0.05, a total of at least 400 randomized patients (200 in each arm) provided the study with 80% power. This is a prespecified secondary end point of the NAPLES II trial.

Continuous variables are given as mean±SD; categorical values are expressed as total number and percentage of the global population (in parentheses). LV indicates left ventricular; ACE, angiotensin-converting enzyme; and PCI, percutaneous coronary intervention.

**Results**

The clinical and biochemical characteristics were well matched between the 2 groups (Tables 1 and 2). sCyC increased significantly more in the control group than in the atorvastatin group (P=0.005; F=5.52 by repeated-measures ANOVA; Figure 2A). CIAKI occurred in 9 of 202 patients in the atorvastatin group (4.5%) and in 37 of 208 patients in the control group (17.8%) (P=0.005; odds ratio=0.22; 95% confidence interval, 0.07–0.69; Figure 2B). sCr increased significantly more in the control group than in the atorvastatin group (P=0.018; F=4.97 by repeated-measures ANOVA). An increase of sCr concentration ≥0.5 mg/dL, at 48 hours from baseline value occurred in 7 of 202 patients (3.5%) in the atorvastatin group and in 16 of 208 patients (7.7%) in the control group and in 16 of 208 patients (7.7%) in the control group.
group (P=0.085). An increase of sCr concentration ≥25% at 48 hours from baseline value occurred in 6 of 202 patients (3%) in the atorvastatin group and in 14 of 208 patients (7%) in the control group (P=0.10) (Figure I in the online-only Data Supplement). We also performed a stratified analysis to determine the benefit of atorvastatin according to the severity of CKD (eGFR ≤30 versus 31–60 mL/min per 1.73 m²) and the presence of diabetes mellitus (Figure 2C). The rate of CIAKI was lower in the atorvastatin group in both diabetics and nondiabetics and in patients with eGFR 31 to 60 mL/min per 1.73 m². On the contrary, no difference was observed in the subgroup with severe CKD (eGFR ≤30 mL/min per 1.73 m²). We observed that neither sCr nor sCyC was altered by administration of a single high (80 mg) atorvastatin loading dose (Figure II in the online-only Data Supplement).

One-year outcome was available in 402 of 410 patients (98%). Clinical and biochemical characteristics of the patients are reported in Tables II and III in the online-only Data Supplement. Major adverse events (including death and dialysis) occurred in 37 of 402 patients (9%). In particular, death occurred in 29 patients (7%) and chronic dialysis in 8 patients (2%). Major adverse events occurred in 9 of 45 patients (20%) with CIAKI (ie, a CyC ≥10% at 24 hours after contrast exposure) and in 28 of 357 patients (7.8%) without CIAKI (P=0.013).

### Atorvastatin Effects on CM-Induced Renal Cell Damage

In both MDCK and HK2 cells exposed to CM, pretreatment with atorvastatin induced an increase in cell vitality and a reduction of cell death (Figure 3). This protective effect was evident after 6 hours and reached a peak at 12 hours of atorvastatin pretreatment. Interestingly, we observed that pretreatment with atorvastatin reduced the CM-induced activation of caspase-3, JNK, and p53 (Figures 4 and 5).

We then evaluated the effects of atorvastatin pretreatment on survival signals mediated by Akt and ERK pathways. CM induced a strong reduction of the phosphorylated (activated) forms of Akt and ERK (Figure 5C and 5D). Interestingly, atorvastatin almost completely restored the survival signal in kidney cells. We performed a further experiment to investigate the effect of 2 hours of NAC pretreatment (100 mmol/L) in the presence of atorvastatin (0.2 μmol/L) on cell death after 3-hour incubation with iodixanol (200 mg/mL). The beneficial effect of the combination of NAC and atorvastatin was higher than that obtained with the NAC or atorvastatin alone (P=0.010; F=10.5 by ANCOVA test; Figures 3C, 3D, and 4B). Finally, we did not observe any involvement of the

---

**Table 2. Biochemical Characteristics of Patients Enrolled in the 2 Groups**

<table>
<thead>
<tr>
<th></th>
<th>Control Group (n=208)</th>
<th>Atorvastatin Group (n=202)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum creatinine, median (range), mg/dL</td>
<td>1.29 (0.88–1.61)</td>
<td>1.32 (0.96–1.62)</td>
</tr>
<tr>
<td>Serum cystatin C, median (range), mg/dL</td>
<td>1.25 (1.0–1.59)</td>
<td>1.23 (1.06–1.62)</td>
</tr>
<tr>
<td>eGFR, mL/min per 1.73 m²</td>
<td>43±14</td>
<td>42±13</td>
</tr>
<tr>
<td>≤30 mL/min per 1.73 m²</td>
<td>38 (18.5)</td>
<td>37 (18.5)</td>
</tr>
<tr>
<td>Contrast nephropathy risk score*</td>
<td>7.5±2.7</td>
<td>8.1±2.8</td>
</tr>
<tr>
<td>Serum urea nitrogen, mg/dL</td>
<td>Baseline 78±31</td>
<td>80±35</td>
</tr>
<tr>
<td></td>
<td>After 48 h 70±30</td>
<td>76±35</td>
</tr>
<tr>
<td>Serum sodium, mEq/L</td>
<td>Baseline 140±5</td>
<td>140±3</td>
</tr>
<tr>
<td></td>
<td>After 48 h 140±5</td>
<td>141±4</td>
</tr>
<tr>
<td>Serum potassium, mEq/L</td>
<td>Baseline 4.7±0.7</td>
<td>4.6±0.7</td>
</tr>
<tr>
<td></td>
<td>After 24 h 4.5±0.7</td>
<td>4.6±0.7</td>
</tr>
</tbody>
</table>

Continuous values are expressed as median and first and third quartiles (serum creatinine and cystatin C) or mean±SD; categorical values are expressed as total number and percentage of the global population (in parentheses). eGFR indicates estimated glomerular filtration rate.

*According to Mehran et al.17

---

**Figure 2. A.** Serum cystatin C concentration at baseline and 24 and 48 hours after contrast media administration in the control group (open symbol, continuous line) and in the atorvastatin group (closed symbol, dashed line); P=0.005; F=5.32 by linear mixed model. **B.** Incidence of contrast-induced acute kidney injury (CIAKI) in control and atorvastatin groups. **C.** Benefit of atorvastatin according to severity of chronic kidney disease (estimated glomerular filtration rate [GFR] ≤30 vs 31–60 mL/min per 1.73 m²) and presence of diabetes mellitus. CI indicates confidence interval.
JAK/STAT pathway in atorvastatin renal cell protection. Indeed, no differences in HSP70 protein (one of the signaling molecules of this pathway) expression have been detected in cells treated with atorvastatin (Figure 5B).

To clarify the clinical impact of these features, we evaluated the activation of JNK, p53, and caspase-3 in epithelial tubular cells collected at 24 and 48 hours after CM exposure from 10 patients (5 in the atorvastatin group and 5 in the control group). The presence of epithelial tubular cells was confirmed by immunohistochemistry by hematoxylin and eosin staining (Figure 7). In controls, an increase of both JNK and p53 phosphorylation (Figure 6B through 6D). Cells collected from patients were also analyzed immunohistochemically with anti-caspase-3 antibodies. Consistent with Western blot data, the activation of pro-caspase-3 at 24 and 48 hours was observed immunohistochemistry by hematoxylin and eosin staining (Figure 7). In controls, an increase of both JNK and p53 phosphorylation (Figure 6B through 6D). Cells collected from patients were also analyzed immunohistochemically with anti-caspase-3 antibodies. Consistent with Western blot data, the activation of pro-caspase-3 at 24 and 48 hours was observed in the control group but not in the atorvastatin group (Figure 7).

## Discussion

### Clinical Findings

The present study demonstrates that a single high (80 mg) loading dose of atorvastatin administered within 24 hours before CM exposure is effective in reducing the rate of CAAKI. This beneficial effect was observed in patients with and without diabetes mellitus as well as in those with moderate CKD (eGFR 31–60 mL/min per 1.73 m²). On the contrary, no advantage was evident in patients with severe CKD (eGFR ≤30 mL/min per 1.73 m²).

At present, the evidence for the use of statins to prevent CAAKI is conflicting and inconclusive. A number of considerations may be involved in the conflicting results. First, the sample size is often modest to detect significant differences in the CAAKI rate. This may often be due to the enrollment of patients at low risk for CAAKI. The lack of observed benefit may therefore represent a type II error (ie, concluding that a benefit does not exist when one really does). Toso et al, for example, did not observe any significant effect of atorvastatin loading dose on the CAAKI rate. However, the 304 patients enrolled in that study were insufficient to detect the expected 50% relative decrease in CAAKI rate in the atorvastatin group (from 15% in the placebo group) with 90% power at the conventional, 2-sided significance level of 5%. Indeed, >350 subjects in each group would have been required to test the hypothesis. Moreover, our study is also underpowered to demonstrate the impact of the atorvastatin loading dose in preventing CAAKI with the use of the current sCr cutoffs. Indeed, with an

Figure 3. Effects of atorvastatin (Ato) (0.2 μmol/L) pretreatment for different times (2, 4, 6, 12, 24, 36 hours) on contrast media-induced Madin Darby distal nonhuman tubular epithelial (MDCK) and human embryonic proximal tubules (HK2) cell damage, assessed as viability (cell titr proliferation assay (A and B) and percentage of apoptotic cells evaluated by fluorescence-activated cell sorting analysis (C and D). Pretreatment with both N-acetylcysteine (NAC) (100 mmol) and atorvastatin (0.2 μmol/L) was more effective than each single compound alone in the prevention of contrast media-induced apoptosis; *P<0.001 vs column (NAC– and atorvastatin–); ‡P<0.03 vs columns (NAC– and atorvastatin+) and (NAC+ and atorvastatin–). All cells were incubated for 3 hours with iodixanol (200 mg iodine/mL). Each experiment was repeated 3 times. In A and B, a mixed linear model for repeated measures was used, with Bonferroni adjustment. In C and D, the Student t test was used.

Figure 4. Effect of atorvastatin (Ato) (0.2 μmol/L) pretreatment on contrast media-induced Madin Darby distal nonhuman tubular epithelial (MDCK) (A) and human embryonic proximal tubules (HK2) (B) cell damage, assessed by caspase-3 assay or by Western blot of pro-caspase-3, *P<0.001 vs control; ‡P<0.001 vs column (iodixanol+ and atorvastatin–). Pretreatment with both N-acetylcysteine (NAC) (100 mmol) and atorvastatin (0.2 μmol/L) was more effective than each single compound alone in the prevention of contrast media-induced caspase-3 activation. *P<0.001 vs column (NAC– and atorvastatin–); ‡P<0.03 vs columns (NAC– and atorvastatin+) and (NAC+ and atorvastatin–). Each experiment was repeated 3 times. The Student t test was used.

Figure 5. Effects of atorvastatin (Ato) (0.2 μmol/L) pretreatment (2, 4, 6, 12, 24, 36 hours) on contrast media-induced Madin Darby distal nonhuman tubular epithelial (MDCK) and human embryonic proximal tubules (HK2) cell damage, assessed as viability (cell titr proliferation assay (A and B) and percentage of apoptotic cells evaluated by fluorescence-activated cell sorting analysis (C and D). Pretreatment with both N-acetylcysteine (NAC) (100 mmol) and atorvastatin (0.2 μmol/L) was more effective than each single compound alone in the prevention of contrast media-induced apoptosis; *P<0.001 vs column (NAC– and atorvastatin–); ‡P<0.03 vs columns (NAC– and atorvastatin+) and (NAC+ and atorvastatin–). All cells were incubated for 3 hours with iodixanol (200 mg iodine/mL). Each experiment was repeated 3 times. In A and B, a mixed linear model for repeated measures was used, with Bonferroni adjustment. In C and D, the Student t test was used.
absolute sCr increase \( \geq 0.5 \text{ mg/dL} \) as primary end point, to demonstrate a reduction from 10% to 5%, \( \approx 1000 \) patients (450 in each arm) would be required.\(^{14}\) In addition, when the \( \geq 25\% \) sCr increase is selected as the primary end point, to demonstrate a reduction of CIAKI from 5% to 2%, \( \approx 1200 \) patients (600 in each arm) would be required.\(^{15,18,24}\) In the present study, we used SCyC as a marker of kidney function to detect CIAKI for several reasons: (1) SCyC is more sensitive than sCr to rapidly detect acute changes in renal function\(^{25,26}\); (2) SCyC allows an early (24 hours) diagnosis of CIAKI\(^{18,27}\); and (3) SCyC predicts the occurrence of major adverse events at follow-up in patients with CKD undergoing CM exposure.\(^{18,20}\)

The second consideration is primary end point definition. In the negative Prevention of Radiocontrast Medium Induced Nephropathy Using Short-Term High-Dose Simvastatin in Patients With Renal Insufficiency Undergoing Coronary Angiography (PROMISS) trial,\(^{11}\) the authors hypothesized an absolute sCr difference between baseline and 48 hours of 0.36 mg/dL in the simvastatin group and 1.1 mg/dL in the control group. This means that, with a baseline sCr level of 1.2 mg/dL, the authors expected a peak increase in the sCr concentration of 28% for the simvastatin group and 92% percent for the control group. Although such a large effect size has been observed in a single-center CIAKI trial,\(^{28}\) multicenter trials generally produce a much smaller effect, with an absolute sCr difference between baseline and 48 hours \( \leq 0.20 \) mg/dL and a peak increase in the sCr concentration of 20% to 30%.\(^{24,25,29–31}\) In addition, the absolute sCr difference is generally not a good primary outcome because it

Figure 5. Western blot analysis showing the effect of atorvastatin (At) \((0.2 \mu\text{mol/L})\) pretreatment on contrast media–induced activation of Jun N-terminal kinase (JNK) and p53 in Madin Darby distal nonhuman tubular epithelial (MDCK) (A) and human embryonic proximal tubules (HK2) (B) renal cells. The activation of JNK and p53 was evaluated by detection of the phosphorylated (activated) form of the proteins (pJNK and pP53\(^{57\text{Ser}}\)). Pretreatment with both N-acetylcysteine (NAC) (100 mmol) and atorvastatin \((0.2 \mu\text{mol/L})\) was more effective than each single compound alone. No differences of HSP70 protein (one of the signaling molecules of the JAK/STAT pathway) expression have been detected in cells treated with atorvastatin (B) in Western blot analysis, showing that atorvastatin \((0.2 \mu\text{mol/L})\) pretreatment induced an increase of the phosphorylated (activated) levels of both Akt and ERK (pAkt and pERK) in the presence of iodixanol in MDCK (C) and HK2 (D) cells.

Figure 6. In vivo effects of contrast media on epithelial tubular renal cells. Western blot analysis assessing Jun N-terminal kinase (JNK) and p53 phosphorylation (activation) levels in epithelial tubular cells from 2 patients in the control group (A through C) and 2 patients in the atorvastatin group (B through D) is shown. The analysis revealed that activation of JNK and p53 was higher in the control group than in the atorvastatin group.
has not been validated to predict adverse outcomes (such as CIAKI).

The third consideration is type and dose of statin administered. Although results of retrospective studies (which included patients receiving a multitude of statins) may suggest the presence of a class effect, the majority of prospective randomized trials demonstrating prevention of CIAKI in patients undergoing CM exposure used short-term pretreatment with high doses of atorvastatin. Some evidence also exists on the better prophylactic effect of a high versus a low statin dose. Thus, when a strategy of short-term pretreatment with statins before CM exposure is adopted, it would be appropriate to use potent statins at high doses.

Mechanisms of Prevention of CM-Induced Renal Cell Damage by Atorvastatin

The cornerstone of the prophylaxis of CIAKI is hydration; however, strategies to prevent CM-induced renal cell apoptosis seem to play a clinical role. Previous studies have demonstrated that CM induce an increase in ROS production. This leads to eventual activation of the stress kinases JNK1/2 and p38. For this reason, clinical trials have been performed with the use of various antioxidant compounds with the aim of lowering the occurrence of CIAKI by scavenging ROS. The present study shows the additive protective effect of atorvastatin over the combined administration of sodium bicarbonate solution and NAC; this supports the hypothesis that the combination of different antioxidant compounds seems to be more effective than a single agent in preventing CIAKI. The Acetylcysteine for Prevention of Renal Outcomes in Patients Undergoing Coronary and Peripheral Vascular Angiography (ACT) trial showed no advantages in routine NAC use. However, several aspects need to be addressed before one reaches the strong conclusion that NAC should be abandoned, including baseline CKD severity, consistency of hydration protocol, and impact of CM selection. Indeed, a recent meta-analysis of 30 trials showed a renoprotective benefit with NAC. NAC pretreatment inhibits CM-induced ROS production and therefore inhibits JNK and p38 activation as well as apoptosis, suggesting the existence of a specific target for NAC upstream of the apoptosis-executing stress kinases in the CM-activated signaling pathway. Atorvastatin may work at a different level in preventing activation of the intrinsic apoptotic pathway. Statins reduces the intracellular ROS levels in many cellular systems by acting on the inhibition of ROS-producing enzymes. In our in vitro model, pretreatment with a high dose of atorvastatin reduced contrast-induced JNK activation, which therefore led to intrinsic apoptosis pathway activation. On the contrary, activation of the JAK2/STAT5 pathway does not seem to have a role in the protective effect of atorvastatin on contrast-induced renal cell damage; indeed, unlike asialo-erythropoietin, atorvastatin does not induce an increase in HSP70 cellular levels. Atorvastatin induces an increase in the survival signals and a reduction of the death signals mediated by CM treatment of kidney cells. This effect was time dependent, reaching a maximum effect at 12 hours of statin incubation. We also, for the first time, confirmed in vivo these mechanisms. In vitro studies addressing the pathophysiology of CM-induced apoptosis have been criticized because of several limitations, including the following: (1) assessment of only 1 potential mechanism of CM-induced renal cell damage in the absence of confounding variables that can be found in vivo (eg, hypoxia due to hemodynamic changes or other systemic mechanisms); (2) exposure to a constant concentration of CM to all cell lines, whereas in vivo the more distal epithelial tubular cells are exposed to much higher concentrations; (3) the potentially high dose of CM; and (4) differences in the tested drug/compound metabolism and transport across cell membranes. Cultured cells are attached with their basolateral membrane to the culture dish. This may preclude the access of atorvastatin to the cells through the active mechanism because the organic amino-transporting polypeptide is mainly present on the basolateral side of the epithelial renal cells. Of note, however, the cytochrome 3A4, which metabolizes atorvastatin into active...
metabolites, is consistently expressed in proximal tubular epithelial cells. Moreover, several studies indicate that the parent drug is equipotent to the active metabolites in vitro.

**Study Limitations**

The present study is a prespecified secondary end point of the NAPLES II trial, which was designed to assess whether a single, high (80 mg), loading (within 24 hours) dose of atorvastatin is effective in preventing elevation of biomarkers of myocardial infarction after elective coronary stent implantation. The lack of randomization of the patients with CKD may represent a limitation. However, the most important characteristics of the 2 groups were well balanced, without significant differences. The present study was powered with sCyC as a marker of kidney damage. The current gold standard for kidney function is still sCr. Having the sCr cutoffs as primary end points would have required a much larger (>1000 patients) sample size to detect the beneficial prophylactic effect of atorvastatin. Moreover, serum CyC is a sensitive marker for predicting the occurrence of major adverse cardiovascular events. Finally, the 4% loss to follow-up rate should be taken into account when our results are interpreted. Indeed, although the 17 patients lost at follow-up were largely similar to those analyzed (online-only Data Supplement), every patient lost to follow-up can be considered a potential threat to robust and precise inference.

**Conclusions**

A single high loading dose of atorvastatin administered within 24 hours before CM exposure is effective in reducing the rate of CIAKI by preventing CM-induced epithelial tubular renal cell apoptosis and increasing the survival signaling pathways. The advantage of adding an atorvastatin loading dose to the sodium bicarbonate solution and NAC seems to be effective in patients at low to medium risk but not in those at high risk.

**Sources of Funding**

This work was supported in part by funds from Associazione Italiana Ricerca sul Cancro (grant 10620) to Dr Condorelli, MERIT (RBNE08ESCZ 002) to Dr Condorelli, and PON01-02342 to Dr Briguori. Dr Quintavalle is a recipient of a Federazione Italiana Ricerca sul Cancro (grant 10620) to Dr Condorelli, MERIT fellowship. Dr De Micco is recipient of a PON01-02342 fellowship. Dr De Micco is recipient of a FIRC fellowship. Dr De Micco is recipient of a MERIT fellowship. Dr De Micco is recipient of a FIRC fellowship. Dr De Micco is recipient of a PON01-02342 fellowship. Dr De Micco is recipient of a PON01-02342 fellowship. Dr De Micco is recipient of a PON01-02342 fellowship.

**Disclosures**

None.

**References**

CLINICAL PERSPECTIVE

Patients with chronic kidney disease were randomly assigned to (1) the atorvastatin group (atorvastatin loading dose [80 mg] within 24 hours before contrast media exposure; n = 202) or (2) the control group (n = 208). All patients received a high dose of N-acetylcysteine and sodium bicarbonate solution. Contrast-induced acute kidney injury (defined as an increase >10% of serum cystatin C) occurred in 9 of 202 patients in the atorvastatin group (4.5%) and in 37 of 208 patients in the control group (17.8%) (P = 0.005; odds ratio = 0.22; 95% confidence interval, 0.07–0.69). In the in vitro model, pretreatment with atorvastatin (1) prevented contrast media–induced renal cell apoptosis by reducing activation of stress kinases and (2) restored survival signals (mediated by Akt and ERK pathways). The present study demonstrates that a single high loading dose of atorvastatin administered within 24 hours before contrast media exposure (on top of conventional strategies) is effective in reducing the rate of contrast-induced acute kidney injury by preventing contrast media–induced epithelial tubular renal cell apoptosis and increasing survival signaling pathways.
Impact of a High Loading Dose of Atorvastatin on Contrast-Induced Acute Kidney Injury
Cristina Quintavalle, Danilo Fiore, Francesca De Micco, Gabriella Visconti, Amelia Focaccio, 
Bruno Golia, Bruno Ricciardelli, Elvira Donnarumma, Antonio Bianco, Maria Assunta Zabatta, 
Giancarlo Troncone, Antonio Colombo, Carlo Briguori and Gerolama Condorelli

Circulation. 2012;126:3008-3016; originally published online November 12, 2012; 
doi: 10.1161/CIRCULATIONAHA.112.103317
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2012 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the 
World Wide Web at:
http://circ.ahajournals.org/content/126/25/3008

Data Supplement (unedited) at:
http://circ.ahajournals.org/content/suppl/2012/11/12/CIRCULATIONAHA.112.103317.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published 
in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial 
Office. Once the online version of the published article for which permission is being requested is located, 
click Request Permissions in the middle column of the Web page under Services. Further information about 
this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/
SUPPLEMENTAL MATERIAL

Effect of high loading dose of atorvastatin on serum creatinine concentrations after contrast media exposure. sCr increased significantly more in the Control group than in the Atorvastatin group (p=0.018; F = 4.97 by repeated measure of variance). An increase of sCr concentration ≥0.5 mg/dL at 48 hours from baseline value occurred in 7/202 (3.5%) patients in the Atorvastatin group and in 16/208 patients (7.7%) in the Control group (p = 0.085). An increase of sCr concentration ≥25% at 48 hours from baseline value occurred in 6/202 (3%) patients in the Atorvastatin group and in 14/208 patients (7%) in the Control group (p = 0.10) (Figure 1S).

Figure 1S

A

B

C

p = 0.085

p = 0.10
Effect of high loading dose of atorvastatin on serum creatinine and cystatin C concentrations. We analyzed 20 patients with chronic kidney disease and assessed the effects of a single high (80 mg) atorvastatin loading dose on renal function in the absence of contrast media exposure, using two surrogate markers of GFR, serum creatinine and cystatin C. These markers were measured simultaneously before and 24 h and 48 h after atorvastatin administration. These patients were not part of the original NAPLES II trial. This amendment of the NAPLES II trial was approved by our Ethic Committee, and all the 20 patients signed the informed consent. The clinical characteristics of the 20 enrolled patients are summarized in the Table 1S. We observed that neither serum creatinine nor cystatin C was altered by administration of a single high (80 mg) atorvastatin loading dose (Figure 2S).

Table 1S. Clinical characteristics of the 20 patients.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>75±11</td>
</tr>
<tr>
<td>Male</td>
<td>10 (50%)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28±6</td>
</tr>
<tr>
<td>sCr, mg/dL</td>
<td>1.45 (1.06-1.67)</td>
</tr>
<tr>
<td>eGFR, ml/min/1.73 m²</td>
<td>43±9</td>
</tr>
<tr>
<td>sCyC, mg/dL</td>
<td>1.48 (1.30-1.80)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>9 (45%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>15 (75%)</td>
</tr>
<tr>
<td>LV ejection fraction, %</td>
<td>55±7</td>
</tr>
<tr>
<td>Drugs:</td>
<td></td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>8 (40%)</td>
</tr>
<tr>
<td>Calcium channel blocker</td>
<td>6 (30%)</td>
</tr>
<tr>
<td>Angiotensin II receptor inhibitor</td>
<td>6 (30%)</td>
</tr>
<tr>
<td>Diuretics</td>
<td>10 (50%)</td>
</tr>
<tr>
<td>Beta blockers</td>
<td>14 (70%)</td>
</tr>
</tbody>
</table>

BMI = body mass index. sCr = serum creatinine; eGFR = estimated glomerular filtration rate; sCyC = serum cystatin C; LV = left ventricular. sCr and sCyC are expressed as median and Q1-Q3.
*p >0.05 by paired t test versus baseline.
Patients lost at follow-up.

Table 2S. Clinical characteristics of the patients lost at follow-up

<table>
<thead>
<tr>
<th></th>
<th>Patients included (n= 410)</th>
<th>Patients lost at follow-up (n= 17)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>70 ± 9</td>
<td>68 ± 6</td>
<td>0.53</td>
</tr>
<tr>
<td>Male</td>
<td>223 (54%)</td>
<td>10 (59%)</td>
<td>0.80</td>
</tr>
<tr>
<td>Body-mass index (kg/m²)</td>
<td>28±5</td>
<td>27±2</td>
<td>0.37</td>
</tr>
<tr>
<td>LV ejection fraction (%)</td>
<td>50±9</td>
<td>51±7</td>
<td>0.58</td>
</tr>
<tr>
<td>Systemic Hypertension</td>
<td>354 (86%)</td>
<td>15 (88%)</td>
<td>0.95</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>169 (41%)</td>
<td>7 (41%)</td>
<td>0.80</td>
</tr>
<tr>
<td>Volume of contrast media (ml)</td>
<td>180±76</td>
<td>171±49</td>
<td>0.51</td>
</tr>
<tr>
<td>Contrast ratio &gt;1</td>
<td>106 (26%)</td>
<td>4 (23%)</td>
<td>0.96</td>
</tr>
</tbody>
</table>

LV = left ventricular; Continuous values are expressed as mean ± standard deviation; categorical values are expressed as a total number and as a percentage of the global population (in parenthesis).

Table 3S. Clinical characteristics of the patients lost at follow-up

<table>
<thead>
<tr>
<th></th>
<th>Patients included (n= 410)</th>
<th>Patients lost at follow-up (n= 17)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum creatinine, median (range), mg/dl</td>
<td>1.30 (0.88-1.62)</td>
<td>1.30 (1.20-1.45)</td>
<td>0.56</td>
</tr>
<tr>
<td>Serum cystatin C, median (range), mg/dl</td>
<td>1.25 1.0-1.62)</td>
<td>1.22 (1.02-1.55)</td>
<td>0.56</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73 m²)</td>
<td>43 ± 14</td>
<td>45 ± 8</td>
<td>0.54</td>
</tr>
<tr>
<td>Contrast nephropathy risk score*</td>
<td>7.8 ± 2.7</td>
<td>8.1 ± 2.8</td>
<td>0.65</td>
</tr>
</tbody>
</table>

eGFR = estimated glomerular filtration rate. *According to Mehran et al. 7. Continuous values are expressed as median and first and third quartiles (serum creatinine and cystatin C) or mean ± standard deviation; categorical values are expressed as a total number and as a percentage of the global population (in parenthesis).
FIGURE LEGENDS Supplement

Figure 1S. Panel A: serum creatinine concentration at baseline, 24 and 48 hours after contrast media administration in the Control group (open symbol, continuous line) and in the Atorvastatin group (closed symbol, dashed line); Panel B: incidence of contrast-induced-acute kidney injury (CIAKI; defined as a serum creatinine increase ≥0.5 mg/dl at 48 hours) in the Control group and in the Atorvastatin group. Panel C: incidence of contrast-induced-acute kidney injury (CIAKI; defined as a serum creatinine increase ≥25% at 48 hours) in the Control group and in the Atorvastatin group.

Figure 2S. Serum creatinine (panel A) and cystatin C (panel B) concentrations at baseline and at 24 and 48 hours after a single high (80 mg) loading dose of atorvastatin in patients with chronic kidney disease. *p >0.05 by paired t test versus baseline.