Kidney International, Vol. 64 (2003), pp. 281–289

Toxic acute tubular necrosis following treatment with zoledronate (Zometa)

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Toxic acute tubular necrosis following treatment with zoledronate (Zometa).

Background. Renal failure and toxic acute tubular necrosis (ATN) may be seen following exposure to a variety of therapeutic agents. Zoledronate (Zometa) is a new, highly potent bisphosphonate used in the treatment of hypercalcemia of malignancy. We report the first clinical-pathologic study of nephrotoxicity associated with this agent.

Methods. A cohort of six patients (four males and two females) with a mean age of 69.2 years received bisphosphonate therapy for multiple myeloma (five patients) or Paget's disease (one patient). In all patients, zoledronate was administered at a dose of 4 mg intravenously monthly, infused over at least 15 minutes, and the duration of therapy was mean 4.7 months (range, 3 to 9 months).

Results. All patients developed renal failure with a rise in serum creatinine from a mean baseline level of 1.4 mg/dL to 3.4 mg/dL. Renal biopsy revealed toxic ATN, characterized by tubular cell degeneration, loss of brush border, and apoptosis. Immunohistochemical staining revealed a marked increase in cell cycle-engaged cells (Ki-67 positive) and derangement in tubular Na^+,K^+-ATPase expression. Importantly, although all patients had been treated with pamidronate prior to zoledronate, no biopsy exhibited the characteristic pattern of collapsing focal segmental glomerulosclerosis observed in pamidronate nephrotoxicity. Following renal biopsy, treatment with zoledronate was discontinued and all six patients had a subsequent improvement in renal function (mean final serum creatinine, 2.3 mg/dL at 1 to 4 months of follow-up).

Conclusion. The close temporal relationship between zoledronate administration and the onset of renal failure and the partial recovery of renal function following drug withdrawal strongly implicate this important and widely used agent in the development of toxic ATN.

Key words: zoledronate (Zometa), acute renal failure, nephrotoxicity, toxic acute tubular necrosis.

Received for publication November 19, 2002
and in revised form January 6, 2002, and February 18, 2003
Accepted for publication March 4, 2003

Toxic tubular injury may follow administration of a variety of therapeutic or diagnostic agents, most notably antineoplastic agents such as cisplatin, the aminoglycoside antibiotics, amphotericin B, and radiocontrast agents. Pathologic evaluation reveals widespread tubular degenerative changes in the absence of glomerular pathology, interstitial nephritis, or vascular disease. Toxic acute tubular necrosis (ATN) must be differentiated from other forms of drug-induced toxicity targeting other compartments of the kidney, such as minimal change disease secondary to nonsteroidal anti-inflammatory drugs (NSAIDs), acute interstitial nephritis secondary to beta lactam antibiotics, and hyaline or thrombotic arteriolopathy secondary to calcineurin inhibitors. We recently reported the occurrence of collapsing focal segmental glomerulosclerosis following treatment with high-dose pamidronate, a bisphosphonate used in the treatment of hypercalcemia of malignancy [1].

Zoledronate (Zometa; Novartis Pharmaceuticals, East Hanover, NJ, USA) is a newer, more potent bisphosphonate that is in widespread use for the treatment of hypercalcemia of malignancy [2, 3]. Deterioration in renal function is a poorly characterized complication of treatment with this agent. We report the occurrence of renal failure secondary to toxic ATN following treatment with zoledronate. The close temporal association, dosage history, and pathologic findings strongly implicate zoledronate as the inciting agent.

CLINICAL HISTORIES

Case #1

A 59-year-old Caucasian male was diagnosed with immunoglobulin G (IgG) kappa multiple myeloma in 2000 based on bone marrow biopsy and multiple lytic lesions

on skeletal survey. The patient initially was treated with three doses of vincristine, adriamycin, and dexamethasone (VAD) from August to November 2000, which produced a transient decline in his paraprotein. Subsequently, the patient received a single pretransplantation bone marrow mobilization treatment with cytoxan, dexamethasone, etopiside, and cisplatin (DCEP) in December 2000 and stem cell transplantation with pretransplant melphalan in January 2001. The patient received no further treatment for his malignancy since that time other than bisphosphonate therapy. He received pamidronate (90 mg intravenously monthly) from September 2000 until October 2001 and subsequently was switched to zoledronate (4 mg intravenously monthly). Past medical history is significant for long-standing hypertension for 35 years and hyperlipidemia. His medications included zoledronate (4 mg intravenously monthly), amlodipine (5 mg every day), coumadin (5 mg/7.5 mg on alternating days), and simvastatin (20 mg every day). The patient had long-standing renal insufficiency, presumably due to hypertension, with a serum creatinine of 1.5 mg/dL in January 1980, 1.9 mg/dL in 1983, 2.1 mg/dL in 1996, and 1.9 mg/dL in October 2001 when he was switched from pamidronate to zoledronate. Four months later, in January 2002, following four treatments with zoledronate, his creatinine increased to 4.0 mg/dL with a 24-hour urine protein of 1.080 g/day. Renal biopsy was performed. Following the biopsy, treatment with zoledronate was discontinued. Four months later, the patient's serum creatinine declined to 2.4 mg/dL.

Case #2

A 73-year-old Caucasian female was admitted in April 2002 for malaise, anorexia, weight loss, and acute renal failure. Past medical history was significant for Paget's disease for 3 years, breast carcinoma status postmastectomy 5 years ago and currently in remission, hypertension for 1 year, anemia, depression, hypothyroidism, and mild congestive heart failure. The patient's medications included furosemide (40 mg every day), simvastatin (20 mg every day), amlodipine (5 mg every day), oxycodone (40 mg every day), levothyroxine (100 mg every day), lisinopril (5 mg every day), sertraline (50 mg every day), and monthly infusions of zoledronate. The patient previously had been treated for Paget's disease with pamidronate (90 mg once a month) from January 2000 until October 2001. She was then switched to zoledronate (4 mg once a month), receiving a total of four doses between November 27, 2001, and April 5, 2002. The patient had a serum creatinine of 1.4 mg/dL in October 2001 and 1.5 mg/dL in December 2001, with a sharp increase to 3.8 mg/dL by April 2002. Laboratory evaluation disclosed serum calcium 9.1 mg/dL, albumin 3.5 gm/dL, 24-hour urine protein 2 g/day, and hematocrit 24.6%. Urinalysis revealed 3+ protein and an inactive sediment. Renal biopsy was performed on April 17, 2002. Following the biopsy, treatment with zoledronate was discontinued. By May 5, 2002, the creatinine had fallen to 2.6 mg/dL and it remained stable at 2.6 mg/dL on July 20, 2002.

Case #3

A 57-year-old Caucasian female was diagnosed with IgA lambda multiple myeloma in 1997 and subsequently treated with radiation therapy and vertebral stabilization in 1997, allotransplantation in 1998, dexamethasone and additional radiation therapy in 1999, thalidomide briefly in 2000, and bisphosphonates. The patient was initially treated with pamidronate at 90 mg intravenously monthly from December 1997 until September 2001 when she was switched to zoledronate. At that time, the patient had a serum creatinine of 1.3 mg/dL and a 24-hour urine protein of 194 mg/day. Zoledronate was administered at a monthly dose of 4 mg from October 2001 until June 2002 when she was found to have a creatinine of 2.5 mg/dL, 24-hour urine protein 1.3 g/day, and albumin 4.2 gm/dL. The patient's medications included bisoprolol (5 mg every day), atorvastatin (10 mg every day), and zoledronate (4 mg intravenously monthly). A renal biopsy was performed on June 28, 2002. Following the biopsy, treatment with zoledronate was discontinued. Four months later, the patient had a creatinine of 2.3 mg/dL.

Case #4

A 75-year-old Caucasian male presented with pneumonia and subsequently was diagnosed with multiple myeloma in September 1998. The diagnosis of myeloma was based on the presence of monoclonal serum and urine spikes and a bone marrow biopsy revealing greater than 30% plasmacytosis. The patient was treated with melphalan and prednisone from October 1998 to January 1999 and then thalidomide every day from December 2001 until August 2002. Past medical history was significant for long-standing hypertension and coronary artery disease requiring coronary artery bypass graft in 1996. The patient also had been treated for multiple myeloma with bisphosphonates. Initial therapy consisted of pamidronate (90 mg intravenously), which he received for 22 months, discontinuing in September 2001. In October 2001, the patient had a baseline creatinine of 1.4 mg/dL. Treatment with zoledronate commenced in December 2001 and was administered at a dose of 4 mg intravenously. The patient's creatinine increased to 1.6 mg/dL in January 2002 and 1.7 mg/dL in March 2002. At this time, following the fourth dose, zoledronate was discontinued. Nonetheless, his creatinine continued to increase to 2.1 mg/dL in April 2002 and 2.6 mg/dL in May 2002, after which time it slowly declined to a level of 2.0 mg/dL on June 26, 2002. Urinalysis revealed the absence of protein and a bland sediment. Serum and urine protein electrophoresis revealed no evidence of a monoclonal

spike. The patient's medications included erythropoietin (40,000 units/week as required), diltiazem (120 mg every day), zestril (20 mg every day), hydrochlorothiazide (25 mg every day), digoxin (0.125 mg every day), coumadin (warfarin; 5 mg every day), and thalidomide (200 mg every day). Renal biopsy was performed on August 8, 2002, at which time the patient had reached a new nadir creatinine of 1.6 mg/dL.

Case #5

An 85-year-old Hispanic male was diagnosed with multiple myeloma in July 2001 following presentation with lytic lesions of the cervical and thoracic spine (C2 and T8) and with subsequent bone marrow biopsy demonstrating 50% plasmacytosis. Past medical history was significant for long-standing hypertension (25 years' duration), multifocal premature ventricular contractions, congestive heart failure, diet-controlled diabetes mellitus (3 years' duration), arthritis, hypercholesterolemia, mild dementia with depression, and a monoclonal gammopathy of unknown significance. The patient was treated with cyclophosphamide for multiple myeloma from September 2001 to April 2002. He also received two doses of pamidronate (90 mg intravenously) in November 2001 and February 2002 and was then switched to zoledronate (4 mg intravenously), receiving single doses in February, March, and on April 8, 2002. The patient had a serum creatinine of 1.5 mg/dL in October 2001, 1.6 mg/dL in February 2002, and 3.8 mg/dL on April 8, 2002. The patient's medications at that time included metoprolol (100 mg every day), isosorbide mononitrate (30 mg every day), paroxetine (10 mg every day), aspirin (81 mg every day), simvastatin (20 mg every day), ranitidine (150 mg every day), furosemide (40 mg twice a day), and ibuprofen (600 mg every day). In response to the increase in serum creatinine, treatment with ibuprofen, zoledronate, and zestril were discontinued. One month later, the patient's serum creatinine further increased to 5.5 mg/dL. Urinalysis revealed 1+ protein and a bland sediment. Urine protein electrophoresis disclosed light chains in the urine and 24-hour urine protein was 1.7 g/day. Renal biopsy was performed on May 7, 2002. Eleven weeks later, the serum creatinine declined to 3.0 mg/dL and persisted at that level at 4 months postbiopsy; during the intervening time period the patient received no additional chemotherapy.

Case #6

A 66-year-old Caucasian man presented with chest pain in September 2001 and was found to have multiple lytic rib lesions. Bone marrow biopsy confirmed the presence of multiple myeloma. Past medical history was significant for hypertension, type 2 diabetes mellitus (with proliferative retinopathy), and carcinoma of the prostate. The patient was initially treated for myeloma with 90 mg pamidronate intravenously once a month, dexamethasone (40 mg intravenously) once a month for 4 months, and thalidomide (100 mg every day). In May 2002, the patient developed unstable angina and coronary artery bypass grafting was performed. At the same time, treatment with pamidronate was discontinued and replaced by zoledronate (4 mg intravenously monthly). Zoledronate was administered monthly from June to September 2002. The patient had a creatinine of 1.0 mg/dL in June 2002, 1.4 mg/dL in July 2002, 1.6 mg/dL in September 2002, and 2.0 mg/dL in October 2002. At that time, the patient had a 24-hour urine protein of 2.6 g/day; urine electrophoresis showed predominantly albumin. Renal biopsy was performed on October 2, 2002. His medications at the time included metoprolol XL (75 mg every day), famotidine (20 mg every day), atorvastatin (10 mg every day), warfarin (5 mg every day), glipizide XL (10 mg every day), pioglitazone (20 mg every day), ramipril (5 mg every day), monthly zoledronate, and thalidomide (100 mg every day). Following renal biopsy, the patient did not receive additional treatment with zoledronate. One month postbiopsy the patient had a serum creatinine of 1.7 mg/dL.

METHODS

All six patients were diagnosed and treated for multiple myeloma or Paget's disease at three different institutions located in New Jersey (four patients) and New York (two patients). All six renal biopsies were processed at Columbia Presbyterian Medical Center for light microscopy, immunofluorescence, and electron microscopy according to standard techniques. For immunofluorescence, 3 um cryostat sections were stained with polyclonal fluorescein isothiocyanate (FITC)-conjugated antibodies to IgG, IgM, IgA, C3, C1q, kappa, lambda, fibrinogen, and albumin (Dako Corporation, Carpenteria, CA, USA).

Immunohistochemical staining was performed on all six cases and compared to three normal controls (normal renal biopsies from patients with isolated hematuria). Staining for Ki-67 (1:40; Dako Corporation) was performed with a Dako autostainer using the Dako Envision Plus detection system (Dako), horseradish peroxidase, and diaminobenzidine substrate. Immunostaining for the alpha subunit of Na^+,K^+-ATPase was performed using avidin-biotin peroxidase technique (Vector Laboratories, Burlingame, CA, USA) as previously described [4]. Briefly, paraffin sections were predigested by microwaving for 17 minutes, incubated overnight with antibody to Na^+,K^+-ATPase (1:10; Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA), overlaid with horse anti-mouse antibody, and developed with diaminobenzidine.

Patients' charts were reviewed for age, gender, race, indication for bisphosphonate therapy, history of onco-

Table 1. Clinical parameters in patients with zoledronate-associated acute tubular necrosis (ATN)

Case number	1	2	3	4	5	6
Age	59	73	57	75	85	66
Gender	Male	Female	Female	Male	Male	Male
Race	C	C	C	C	H	C
Indication for bisphosphonate therapy	MM	Paget's disease	MM	MM	MM	MM
Oncologic treatment						
Total body irradiation	No	No	Yes	No	No	No
Stem cell transplant	Yes	No	Yes	No	No	No
Cisplatin	Single dose	No	No	No	No	No
Pamidronate	Yes	Yes	Yes	Yes	Yes	Yes
Duration of therapy *months*	11	21	46	22	2	8
Maximal dosage *mg/month*	90 mg	90 mg	90 mg	90 mg	90 mg	90 mg
Zoledronate acid						
Duration of therapy *months*	4	4	9	4	3	4
Dose *mg/month*	4	4	4	4	4	4
Infusion time *minutes*	20	15	20	20	15	15
Serum creatinine baseline[a] *mg/dL*	1.9	1.4	1.3	1.4	1.6	1
Renal status at biopsy						
Serum creatinine *mg/dL*	4	3.8	2.5	2.6[b]	5.5	2
24-hour urine protein *g/day*	1.08	2	1.3	Negative	1.7	2.6
Length of post-biopsy follow-up *months*	4	3	4	3	4	1
Final serum creatinine *mg/dL*	2.4	2.6	2.3	1.6	3	1.7
Zoledronate acid discontinued?	Yes	Yes	Yes	Yes	Yes	Yes

Abbreviations are: C, Caucasian; H, Hispanic; MM, multiple myeloma.
[a]Prior to zoledronate administration
[b]Case #4 had a serum creatinine of 1.7 mg/dL after treatment with zoledronate, 2.6 mg/dL 2 months later, and 1.6 mg/dL 5 months posttreatment (at the time of biopsy)

logic treatments, and parameters of renal function. Renal failure was defined as an increase in serum creatinine of at least 1.0 mg/dL or doubling of serum creatinine. In each case, an attempt was made to document and correlate the timing of drug exposures with the development of renal failure due to toxic ATN.

RESULTS

The clinical features of the six patients with renal failure following treatment with zoledronate are summarized in Table 1. The cohort consisted of four males and two females with a mean age of 69.2 years (range, 57 to 85 years). The patients were predominantly Caucasian (83%) and indications for bisphosphonate therapy included multiple myeloma in five patients and Paget's disease in one. Oncologic treatments included stem cell transplantation in two patients, total body irradiation in one patient, and cisplatin (single dose only) in one patient. Of note, all six patients had received pamidronate (90 mg intravenously) once a month for a mean 18.3 months (range, 2 to 46 months) prior to initiation of treatment with zoledronate.

All six patients were treated with zoledronate at the recommended dose of 4 mg intravenously once a month, infused over at least 15 minutes. The duration of therapy with zoledronate prior to renal biopsy ranged from 3 to 9 months (mean, 4.7 months). Prior to treatment with zoledronate, the cohort had a mean serum creatinine of 1.4 mg/dL, including a single patient with creatinine 1.9 mg/dL associated with a 35-year history of hypertension. Following treatment with zoledronate, the mean peak serum creatinine was 3.4 mg/dL, including five patients with doubling of serum creatinine and a single case with an increase in creatinine from 1.3 to 2.5 mg/dL.

Due to the temporal relationship between administration of zoledronate and development of renal failure, zoledronate was discontinued following renal biopsy in five patients and prior to biopsy in a single case (case #4). In all cases, the serum creatinine subsequently declined but did not return to baseline levels in the relatively short follow-up period of 1 to 4 months.

The renal biopsy findings in patients with renal failure following treatment with zoledronate are highlighted in Table 2. Sampling for light microscopy was adequate in all biopsies, with a mean glomerular count of 26.7 (range, 7 to 58 glomeruli). While all biopsies displayed some degree of global glomerulosclerosis, importantly, no case exhibited lesions of focal segmental glomerulosclerosis, a finding that may be seen following treatment with pamidronate [1].

The predominant finding in all six biopsy samples was widespread, marked tubular degenerative changes characterized by luminal ectasia, cytoplasmic simplification with irregular luminal contours, coarse cytoplasmic vacuolization, hypereosinophilia, loss of brush border, enlarged hyperchromatic often atypical nuclei with prominent nucleoli, and apoptotic and mitotic figures (Fig. 1 A and B). Of note, the degree of tubular injury was milder in case #4 in whom treatment with zoledronate

Table 2. Pathologic findings in patients with zoledronate-associated acute tubular necrosis (ATN)

Case number	1	2	3	4	5	6
No. of glomeruli	14	22	40	7	58	19
No. with global GS	1	1	19	2	14	4
No. with focal segmental GS	0	0	0	0	0	0
Tubular degeneration	Diffuse	Diffuse	Diffuse	Diffuse (mild)	Diffuse	Diffuse
TA and IF	Mild	Moderate	Mild	Moderate	Moderate	Mild
Interstitial inflammation	Mild	Moderate	Mild	Mild	Mild	Mild
Vascular disease	Severe	Moderate	Mild	Moderate	Moderate	Severe
PCD-associated disease	No	No	No	No	Yes—MCN & LCDD	No
Immunofluorescence	Negative	Negative	Negative	Negative	Lambda 3+	Negative
EM deposits	No	No	No	No	GBM, MES, TBM	No
EM foot process fusion	25%	20%	NA	10%	15%	50%
Diagnosis—Final	1. ATN 2. HTN	ATN	ATN	ATN	1. ATN 2. MCN 3. LCDD, mild	1. ATN 2. NDGS

Abbreviations are: GS, glomerulosclerosis; TA and IF, tubular atrophy and inerstitial fibrosis; PCD, plasma cell dyscrasia; EM, electron microscopy; MCN, myeloma cast nephropathy; LCDD, light chain deposition disease; GBM, glomerular basement membrane; MES, mesangial; TBM, tubular basement membrane; HTN, hypertensive arterionephrosclerosis; NA, not available; NDGS, nodular diabetic glomerulosclerosis.

had ceased 5 months prior to biopsy. In addition to the diffuse tubular injury, variable degrees of tubular atrophy, interstitial fibrosis, and interstitial inflammation were commonly seen, ranging from mild to moderate in severity. The interstitial inflammation was predominantly confined to zones of tubular atrophy and interstitial fibrosis, and eosinophil infiltration and tubulitis were not identified. There also was mild to moderate vascular disease. The single exception was case #1 that exhibited severe vascular disease associated with a 35-year history of hypertension and a baseline creatinine of 1.9 mg/dL. Case #6 had evidence of coexisting nodular diabetic glomerulosclerosis.

Electron microscopy confirmed the severe tubular degenerative changes. Proximal tubules exhibited epithelial simplification with reduced organellar content, loss or attenuation of brush border, cellular detachment from the tubular basement membrane, apical blebbing, widened intercellular spaces, individual cell necrosis, and focal shedding of cytoplasmic debris into the tubular lumen (Fig. 2).

In five of the six biopsy samples, there was no evidence of myeloma cast nephropathy, amyloidosis, monoclonal immunoglobulin deposition disease, or other form of plasma cell dyscrasia-associated renal disease. In these five biopsies (cases #1 to 4 and 6), immunofluorescence staining was negative and electron microscopy disclosed no electron dense deposits. In contrast, renal biopsy from case #5 exhibited widespread tubular injury similar to the other four cases as well as focal, rare atypical fractured casts, which stained for lambda light chain but not kappa. Based on the findings of widespread tubular injury out of proportion to the rare myeloma casts, as well as the improvement in renal function following discontinuation of zoledronate and without treatment of myeloma, the findings were thought to represent mild myeloma cast nephropathy superimposed on severe zoledronate toxicity. In addition, there were rare granular-powdery electron dense deposits involving the mesangium and glomerular and tubular basement membranes, diagnostic of early light chain deposition disease.

Immunohistochemical staining for Ki-67, a marker of proliferation, was quantitated by counting the number of positively stained tubular nuclei per low power field using a 10× ocular objective; three to five fields were examined per case. Among the three normal controls, positively stained tubular nuclei were seen in mean 3.05 cells per field, compared to mean 30.7 cells per field in the biopsies with zoledronate-associated ATN (Fig. 1C). Of note, the single case of zoledronate-associated ATN in which the drug was withdrawn 5 months prior to biopsy (case #4) exhibited a mean of only 6.0 positively stained tubular nuclei per 10× field.

Immunohistochemical staining for Na^+,K^+-ATPase on normal controls revealed a basolateral distribution of expression with moderate intensity of staining in proximal tubules and more intense staining in distal tubules and medullary thick ascending limbs (Fig. 1D). The expression of Na^+,K^+-ATPase was markedly altered in the setting of zoledronate-associated ATN. In addition to a generalized decrease in intensity of basolateral staining, some tubules exhibited a complete loss of staining or focal apical translocation of Na^+,K^+-ATPase (Fig. 1E). Of note, the altered expression of Na^+,K^+-ATPase was least prominent in case #4 (who had discontinued treatment with zoledronate 5 months prior to biopsy).

DISCUSSION

Zoledronate, a member of the bisphosphonate class of drugs, is an inhibitor of bone resorption. Similar to other bisphosphonates, its mechanism of action is com-

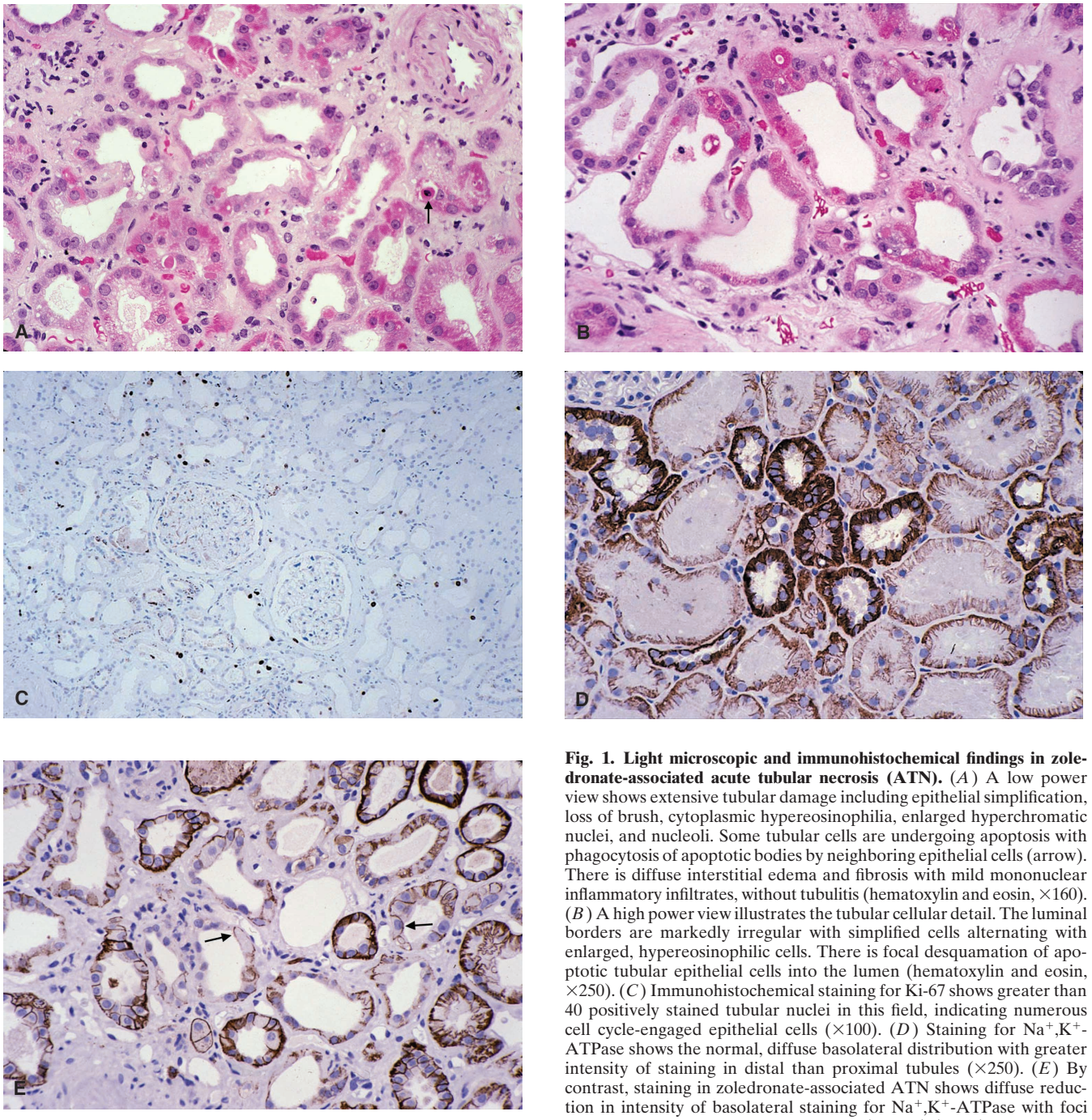

Fig. 1. Light microscopic and immunohistochemical findings in zoledronate-associated acute tubular necrosis (ATN). (*A*) A low power view shows extensive tubular damage including epithelial simplification, loss of brush, cytoplasmic hypereosinophilia, enlarged hyperchromatic nuclei, and nucleoli. Some tubular cells are undergoing apoptosis with phagocytosis of apoptotic bodies by neighboring epithelial cells (arrow). There is diffuse interstitial edema and fibrosis with mild mononuclear inflammatory infiltrates, without tubulitis (hematoxylin and eosin, ×160). (*B*) A high power view illustrates the tubular cellular detail. The luminal borders are markedly irregular with simplified cells alternating with enlarged, hypereosinophilic cells. There is focal desquamation of apoptotic tubular epithelial cells into the lumen (hematoxylin and eosin, ×250). (*C*) Immunohistochemical staining for Ki-67 shows greater than 40 positively stained tubular nuclei in this field, indicating numerous cell cycle-engaged epithelial cells (×100). (*D*) Staining for Na^+,K^+-ATPase shows the normal, diffuse basolateral distribution with greater intensity of staining in distal than proximal tubules (×250). (*E*) By contrast, staining in zoledronate-associated ATN shows diffuse reduction in intensity of basolateral staining for Na^+,K^+-ATPase with foci of complete loss or apical translocation (arrows) (×250).

plex and involves inhibition of bone dissolution by both direct calcium chelation and cellular effects on the osteoclast [5]. Zoledronate is indicated for the treatment of hypercalcemia of malignancy and is effective in the treatment of Paget's disease [6].

Zoledronate was initially developed in an attempt to synthesize a more potent, but less toxic, bisphosphonate with greater ability to inhibit bone resorption. Zoledronate differs from pamidronate in the substitution of an imidazole ring for an amino group in the R^2 side chain. Zoledronate initially was compared to six available bisphosphonates and five additional preclinical compounds in two rat models [7]. First, zoledronate was found to have substantially greater antiresorptive capacity, similar renal tolerabilility, and therefore superior therapeutic ratio to 11 other agents tested in thyroparathyroidectom-

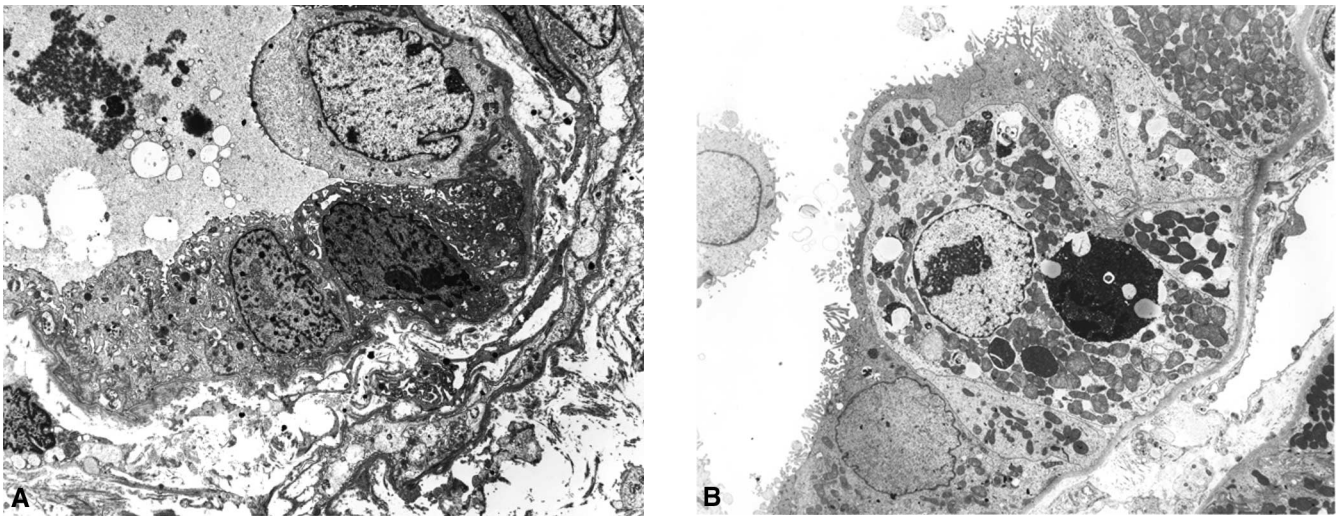

Fig. 2. Ultrastructural findings in zoledronate-associated acute tubular necrosis (ATN). (*A*) Damaged tubules exhibit complete loss of brush border, increased nuclear:cytoplasmic ratio with focal nucleoli, and shedding of cytoplasmic debris into the tubular lumen (×2000). (*B*) An apoptotic body is seen phagocytosed by the tubular epithelial cell at center. The brush border has been lost and the cell is covered by over-hanging cytoplasm from the adjacent cells. A desquamated, degenerating epithelial cell is seen in the lumen (×2000).

ized rats [7]. Second, single 1-hour infusions of varying doses of either zoledronate or pamidronate were compared with respect to renal tolerability. Pamidronate was found to be more nephrotoxic, requiring 10 mg/kg to increase the serum urea nitrogen by 100% at 4 hours, as opposed to 38 mg/kg for zoledronate [7–8]. An important finding was the lack of correlation between antiresorptive capacity and renal tolerability, indicating a dissociation between the two effects [7].

The efficacy of zoledronate in patients with hypercalcemia of malignancy is well established and appears to be superior to pamidronate [2, 3]. Pooled data from two randomized, double-blind studies involving 287 patients and comparing a single dose of zoledronate (4 mg intravenously) to pamidronate (90 mg intravenously) found that zoledronate produced more frequent normalization of serum calcium, longer time to relapse of hypercalcemia, and a quicker rate of action [2]. A phase III, double-blind comparative trial involving 1648 patients with multiple myeloma or advanced breast cancer who were treated with zoledronate or pamidronate every 3 to 4 weeks for 1 year found a greater clinical benefit of zoledronate as measured by the event rate for radiation therapy to bone and the time to first radiation therapy [3].

Renal toxicity has been identified as a complication of treatment with zoledronate. In a phase II double-blind, randomized trial comparing three different doses of zoledronate (administered over 5 minutes) with 90 mg of pamidronate, an increase in serum creatinine of 0.5 mg/dL was seen in 37 of 280 patients (13.2%) [9]. Importantly, this toxicity was seen most commonly at 4 mg, the highest dose tested [9]. The relationship between dosage and renal toxicity is supported by the absence of renal toxicity in a larger study on 351 postmenopausal women with osteoporosis who received only low-dose zoledronate at cumulative annual doses of 1 to 4 mg [10].

The largest body of data on renal toxicity of zoledronate come from a phase III trial involving 1648 patients with multiple myeloma or advanced breast cancer who were treated with zoledronate or pamidronate every 3 to 4 weeks for 1 year [3]. Patients were treated with either 4 or 8 mg doses of zoledronate over a 5-minute infusion and compared to patients receiving pamidronate (90 mg intravenously) infused over 2 hours. Due to concerns over renal toxicity of zoledronate, the study underwent two protocol adjustments. In the first, the infusion time for zoledronate was increased from 5 to 15 minutes. In the second, patients who were initially treated with 8 mg zoledronate had a reduction in dose to 4 mg. Following the two adjustments, renal toxicity declined, confirming that renal impairment following treatment with zoledronate is dose-dependent and infusion time-dependent. Deterioration in renal function (defined as an increase in serum creatinine of $\geq$0.5 mg/dL) in patients treated with 4 mg zoledronate infused over 15 minutes was seen in 23 of 246 patients (9.3%), which was similar to the 8.1% incidence of deterioration in renal function following treatment with pamidronate. On the basis of these findings, the current recommendation for dosing of zoledronate is 4 mg intravenously once a month infused over at least 15 minutes [11]. Serum creatinine monitoring is recommended prior to each dose of zoledronate and discontinuation of zoledronate is advised following irreversible increases in serum creatinine of 0.5 mg/dL in patients without baseline renal insufficiency and 1.0 mg/dL in patients with previously

established renal insufficiency [11]. Rigorous attention to monitoring of serum creatinine and awareness of the potential nephrotoxicity may avert the development of acute renal failure in patients treated with this agent.

There are multiple lines of evidence that point to zoledronate as the etiologic agent producing ATN in our cohort of patients. First, is the previously established renal toxicity associated with this agent [3, 9]. The renal biopsy findings of diffuse tubular injury in the absence of glomerular abnormalities or significant interstitial inflammation, tubulitis, or eosinophils suggest that zoledronate acts as an epithelial toxin that targets proximal tubules. This is supported by the immunohistochemical findings of a marked increase in cell-cycle engaged (Ki-67 positive) tubular epithelial cells and the marked derangement in tubular Na^+,K^+-ATPase expression.

Temporally, the development of renal failure closely parallels the administration of zoledronate. All six patients had relatively normal or mildly impaired renal function prior to the administration of zoledronate and all developed acute or subacute renal failure following treatment. Importantly, all of the patients experienced improvement in renal function following discontinuation of treatment.

The course of renal failure following treatment with zoledronate was primarily subacute, occurring over a period of months. Although the mean duration of zoledronate therapy prior to the development of renal failure was 4.7 months, this constituted a mean of only 4.7 administrations because the drug is administered once per month. Smaller increments in serum creatinine were noted as early as 1 month in case #4 (with increase in creatinine from 1.4 to 1.6 mg/dL) and in case #6 (with increase in creatinine from 1.0 to 1.4 mg/dL). Such a gradual, subacute onset of renal insufficiency over months has precedents in other forms of toxic acute tubular damage, such as following the administration of the antiviral agent foscarnet, or following chemotherapeutic agent cisplatin, where slow release of tissue-bound cisplatin has been incriminated [12,13]. Interestingly, the morphologic appearance of the tubular damage in zoledronate-associated ATN is also reminiscent of that seen following treatment with foscarnet and cisplatin, including the histologic findings of nucleomegaly, nuclear atypia, and cytoplasmic hypereosinophilia. It is also noteworthy that renal insufficiency continued to worsen for 1 to 2 months following discontinuation of zoledronate, perhaps owing to the slow release of tissue-bound drug or a prolonged period for regeneration of the damaged tubular epithelium. The slow recovery is not surprising given the relatively prolonged time course for the development of the tubular injury following zoledronate exposure.

The level of proteinuria noted in our cohort [range, 0 to 2.6 g (mean, 1.7 g)] is slightly greater than expected for uncomplicated ATN. Whereas other underlying glomerular conditions may have contributed to the proteinuria in case #5 (who had mild light chain deposition disease as well as light chain proteinuria) and case #6 (with nodular diabetic glomerulosclerosis), no preexisting glomerular disease could be detected in the others. Thus, we suspect that proximal tubular dysfunction may have been a source for subnephrotic proteinuria in the remainder, although none had evidence of Fanconi syndrome.

The pattern of renal injury seen following treatment with zoledronate is different from the findings reported following treatment with pamidronate [1]. Treatment with high-dose pamidronate has been associated with collapsing focal segmental glomerulosclerosis, a pattern of renal injury characterized by primary podocyte injury, with altered cell cycle regulation and reversion to an immature cellular phenotype [4]. In patients with pamidronate toxicity, as well as idiopathic collapsing focal segmental glomerulosclerosis, widespread tubular injury accompanies the glomerulopathy. By contrast, in zoledronate-associated renal failure there is toxic tubulopathy without associated glomerular injury as evidenced by the absence of significant proteinuria, the absence of lesions of focal segmental glomerulosclerosis, and the preservation of foot process cytoarchitecture. It is noteworthy that all six cases reported herein were treated with pamidronate, often for prolonged periods, prior to treatment with zoledronate. This observation, together with the mild baseline renal insufficiency prior to zoledronate administration, suggests that pretreatment with pamidronate may in some way potentiate the tubular toxicity of zoledronate. Nevertheless, it is clear from the large phase III trial of zoledronate, which excluded patients who had been treated with alternative bisphosphonates over the previous 12-month period, that even patients without prior pamidronate exposure have a 9.3% incidence of renal functional deterioration following zoledronate therapy [3]. Of note, renal failure also has been reported following treatment with other bisphosphonates, including etidronate [14], clodronate [14], and tiludronate [15].

After reaching the systemic circulation, zoledronate is predominantly excreted unchanged via the kidneys [11]. Renal clearance of bisphosphonates exceeds glomerular filtration rate, indicating active renal transport and secretion [16]. Potential mechanisms of tubular toxicity may involve cellular effects similar to those documented in the osteoclast. As occurs in the osteoclast, bisphosphonates are internalized by the proximal tubular cell. Bisphosphonates are known to exert a number of cellular effects on the osteoclast, including inhibition of the mevalonate pathway required for the postranslational lipid modification of small guanosine triphosphatases (GTPases) [17]. By anchoring the GTPases in cell membranes, lipid prenyl groups ensure the correct subcellular compartmental-

ization and function of GTPases in a variety of cellular processes, including integrin signaling, endosomal trafficking, membrane ruffling, and apoptosis [5, 18–21]. Similar cellular effects may occur within the proximal tubular epithelium. Proximal tubular cells require high adenosine triphosphate (ATP) levels for active ionic transport; bisphosphonates may impair cell energetics by incorporation into ATP analogs with resultant inhibition of ATP-dependent metabolic pathways [22]. Bisphosphonates also have been shown to disrupt the osteoclast cytoskeleton by inhibiting the assembly of actin rings, leading to loss of the osteoclast-ruffled border [23]. Similar effects may account for the loss of brush border in proximal tubules in the setting of zoledronate-associated toxic tubular injury.

Zoledronate is a widely used and highly potent therapeutic agent in the treatment of hypercalcemia of malignancy. Zoledronate should be administered at a maximal dose of 4 mg intravenously infused over at least 15 minutes. At recommended doses, renal failure may occur in a minority of patients and is characterized by toxic tubular injury consistent with ATN. Following discontinuation of treatment, renal function typically improves but may not return to baseline levels. Future studies are needed to assess the efficacy and nephrotoxicity of zoledronate administered at lower doses and following more prolonged infusion times. The list of etiologies of renal failure in patients with multiple myeloma should be expanded to include zoledronate-associated ATN.

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