A Non-Nephrotoxic Gentamicin Congener That Retains Antimicrobial Efficacy

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Aminoglycoside antibiotics, although of major clinical importance in the treatment of serious Gram-negative infections and a potential therapeutic agent in the amelioration of diseases that are characterized by premature stop mutations, are associated with a high incidence of acute renal failure. With the use of HPLC techniques, the four components (congeners) of gentamicin, the most commonly used aminoglycoside, were isolated and characterized. Described here is a congener with minimal cytotoxicity in cell culture and animal studies that retained normal bactericidal properties in both *Bacillus subtilis* and a multidrug-resistant form of *Klebsiella pneumoniae*. Furthermore, in animal studies, this congener failed to induce the functional and pathologic changes that are characteristic of gentamicin nephrotoxicity that is seen with the native compound. Finally, internalization of this non-nephrotoxic component was unaltered, but the subcellular distribution was different from native gentamicin or the other three cytotoxic congeners. These studies have identified a component of the native gentamicin congener mixture that retains its bactericidal properties with minimal or no apparent nephrotoxicity.

Aminoglycoside antibiotics have remained a mainstay for the treatment of Gram-negative infections for >40 yr despite their associated nephrotoxicity. With the dramatic increase in sepsis (1), sepsis-induced acute renal failure (ARF) (2), the lack of new antibiotics, and the emergence of multidrug-resistant bacteria (3,4), the continued and even increased use of aminoglycosides is occurring. Unfortunately, a high percentage of patients who are treated with aminoglycosides develop ARF. In addition, patients with existing kidney, liver, or cardiac diseases have a rate of aminoglycoside-induced ARF as high as 50% (5,6). This increased rate of ARF adds morbidity, mortality, and cost to the adverse effects that are associated with this clinically important but potentially dangerous class of antibiotics. Therefore, understanding the intracellular mechanism of aminoglycoside toxicity may lead to approaches that minimize this barrier to its use. Recent data from several genetic diseases secondary to premature stop mutations indicate that gentamicin suppresses reading of the stop codon, resulting in expression of the full-length protein (7,8).

Data from our laboratory have elucidated a trafficking pathway for gentamicin that is responsible in large part for the associated intracellular pathologies (9). In proximal tubule cells, aminoglycosides are internalized by the multiligand receptor megalin (7,10,11) and sequestered by the proximal tubule epithelial primarily into lysosomes. However, a small percentage of the absorbed gentamicin traffics retrograde via the endocytic pathway through the Golgi complex and into the endoplasmic reticulum. It subsequently is released into the cytosol, freeing it for toxic association with subcellular organelles such as the nucleus, mitochondria, intracellular vesicles, and the inner leaflet of surface membranes (9).

Commercial gentamicin is a mixture of various congeners C1 and C1a and the enantiomers C2 and C2a. Structural differences between them are minor, differing only by a methyl or hydrogen substitution in two R groups on the purpuroasamine residue. In a publication by Kohlhepp et al. (12), the nephrotoxicity of the various congeners originally was investigated. Unfortunately, this work was hampered by technical limitations in the ability to separate the enantiomers C2-C2a as well as cross-contamination of the various purified congeners with other congeners in ranges of 2 to 12%. In addition, the C2a content of these purified components was unknown. In this study, we isolated the individual congeners of gentamicin, including the enantiomers C2 and C2a, using current HPLC techniques. The enantiomer C2 exhibited little cellular toxicity and no nephrotoxicity while maintaining bactericidal efficacy. Results from this study could lead to more widespread use of gentamicin in the form of the isolated C2 congener, especially in high-risk patients. This purified congener also could find long-term daily use in treating genetic diseases that are induced by premature stop codon defects in which the presence of gentamicin induces a read-through of the stop codon in the defective message (7,8).
Materials and Methods

Separation and Characterization of Congeners

Separation of native gentamicin was carried out using a C18 column (250 × 4.60 mm), and the chromatogram was developed using a Waters 600e HPLC pump (Milford, MA). A gradient of 100% of a 0.1% (vol/vol) mixture of tri-fluoro-acetic acid in water to 100% of a 0.1% (vol/vol) mixture of tri-fluoro-acetic acid in acetonitrile was used to develop the chromatogram. Retention times of 12.87, 22.07, 23.84, and 25.85 min were noted for congeners C1, C2, C2a, and C1a, respectively. There was no overlap between adjacent peaks. For all studies, the same lot of gentamicin was used. This ensured that the results obtained were independent of potential alterations in the ratio of gentamicin components.

Lyophilized gentamicin congeners were resuspended in ddH2O to a concentration of 20 mg/ml. Actual concentrations then were determined at the clinical chemistry laboratory of the Roudelush VA Hospital. Discrepancies between perceived and actual concentrations likely are due to the hygroscopic nature of gentamicin; actual measured concentrations were used for all dosing calculations.

Bactericidal assays were conducted using a multidrug-resistant form of Klebsiella pneumoniae that was grown in #11 medium (Difco, Sparks, MD) and Bacillus subtilis that was grown on regular tryptic soy agar medium in large 245 × 245-mm agar plates for at least 24 h. Wells were loaded with 100 µl of native gentamicin or the separated congeners at concentrations of 5 or 10 µg/ml (control wells contained PBS). Plates were examined the next day to determine areas of clearance. Areas that were completely clear were quantified for kill zone areas; area that were slightly more opaque than surrounding areas were deemed inhibition areas. Generally, the B. subtilis presented only kill zones.

Cell Toxicity Assays

LLC-PK1 porcine proximal tubule cells were plated on 18-mm circular coverslips and grown in K-P medium supplemented with 10% FBS (HyClone, Logan, UT) and penicillin streptomycin (Sigma-Aldrich, St. Louis, MO). Upon reaching approximately 40% confluence, cells were incubated in media that contained the various congeners or native gentamicin at a final concentration of 1 mg/ml. After approximately 24 h, the cells were washed once with medium and placed in medium that contained 2.5 µg/ml Hoechst 333424 (Molecular Probes, Eugene, OR) and 0.667 µg/ml propidium iodide (Molecular Probes) for 10 min. The cells then were washed in normal medium and visualized live using confocal microscopy.

In Vivo Nephrotoxicity Assay

Male Sprague-Dawley rats (200 to 250 g; n = 3; Harlan, Indianapolis, IN) received a single daily dose of either native gentamicin or the congener C2 at 100 µg/kg or a normal saline solution intraperitoneally for 6 d. Blood samples were taken initially to establish a baseline and daily up until 24 h after the final injection. Serum creatinine then was measured on a Beckman Creatinine Analyzer 2 (Beckman Instruments, Brea, CA) and reported in mg/dl. All protocols were approved by the Institutional Animal Care and Use Committee.

Histology and Indirect Immunofluorescence Localization in Tissue

After completion of the in vivo nephrotoxicity study, rat kidneys were perfusion-fixed with fresh 4% paraformaldehyde in PBS (pH 7.4). Some of the tissue was embedded in paraffin, and 3-µm sections were cut for histology, stained with hematoxylin and eosin (H&E) or periodic acid-Schiff (PAS), and scored by a pathologist (C.L.P.) who was blinded to the interventions. At least two representative sections of each kidney were examined. Morphologic evaluation of injury was assessed by grading the extent of necrosis of the proximal convoluted tubules as outlined by Jablonski et al. (13). Injury scores were assigned on the basis of the predominant pattern present. For indirect immunofluorescence, 100-µm-thick sections were cut on a vibratome and stained with the monoclonal anti-gentamicin antibody (Biodisgnat International, Saco, ME) and a fluorescein-conjugated secondary antibody (Jackson Immunoresearch, West Grove, PA) and lightly counterstained with Alexa 647-phallaloidin (Molecular Probes) to localize filamentous actin for visualization using confocal microscopy.

Indirect Immunofluorescence Localization in LLC-PK1 Cells

Because of the high degree of cell death that was seen in the previous cytotoxicity assay with the congeners, LLC-PK1 cells were allowed to reach approximately 70% confluence before being incubated in medium that contained 1 mg/ml of either native gentamicin or the congeners. During this period of exposure to native gentamicin or the congeners, lysosomes were labeled by adding a 10,000 molecular weight rhodamine dextran to the culture medium (0.5 mg/ml; Molecular Probes). The cells then were fixed and stained for indirect immunofluorescence of nonglial lysosomes, cytosolic gentamicin was carried out as described previously (9), using a Tyramide Signal Amplification (Molecular Probes).

Microscopy

Histologic sections were viewed with a Widefield Nikon microscope with an attached color CCD camera (Nikon, Melville, NY). Fluorescence images were acquired using a Bio-Rad MRC 1024 confocal microscope (Bio-Rad, Hercules, CA) on a Nikon platform (The Fryer Co., Huntley, IL). Co-localization studies with Texas red dextran, FITC-Helix pomatia, and gentamicin were conducted on a Zeiss 510 confocal microscope with images illuminated and acquired sequentially to eliminate bleed-over emissions from the different fluorescence emission spectra.

Statistical Analyses

T tests were conducted on serum creatinine values between congener C2 and the native compound at the various injection days and on Jablonski score values between congener C2 and the native compound using Excel 2003 (Microsoft Corp., Redmond, WA). Differences in necrosis, apoptosis, and cell density among the various congeners on the human and porcine cell lines were determined using an ANOVA function with Systat 11 software (Systat Software, Point Richmond, CA). Statistical significance was achieved at P ≤ 0.05. All reported values are mean ± SEM.

Results

Individual Congeners of Gentamicin Separated by HPLC Retain Their Bactericidal Properties

To determine whether the various congeners of gentamicin retained their bactericidal properties, we first separated the congeners using HPLC techniques. The congeners eluted off the HPLC column in the order C1, C2, C2a, and C1a with retention times of 12.87, 22.07, 23.84, and 25.85 min, respectively (Figure 1A).

Bacterial assays using B. subtilis demonstrated that all individual congeners retained the bactericidal efficacy of the native
compound (Figure 1B). Kill zone measurements in the agar plates indicated similar clearance areas, between 12 and 15 mm at a concentration of 10 μg/ml. Use of a multidrug-resistant form of *Klebsiella pneumoniae* yielded similar results to the *B. subtilis*. With *K. pneumoniae*, bactericidal efficacy decreased, producing smaller kill zones (between 9 and 11 mm at 10 μg/ml) and the appearance of zones of growth inhibition between 13 and 14 mm that were not seen with *B. subtilis* (histogram, Figure 1B). These kill and inhibition zones were equivalent for the four congeners, as well as for the native commercial mixture.

**Enantiomer C2 Exhibited Reduced Toxicity in LLC-PK1 Cells**

Because all the congeners were found to retain bactericidal activity, we next conducted cytotoxicity assays in culture using...
LLC-PK1 proximal tubule cells to determine the toxicity of each individual congener. The nuclear dyes propidium iodide and Hoechst 33342 were used as markers for necrosis and apoptosis, respectively. In this assay, the cell permeant dye Hoechst 33342 labeled nuclei of all cells with an even distribution (Figure 2A, Blank). Apoptotic cells were discerned from normal cells by condensation of nuclear material and increased localized fluorescence (Figure 2A, Native, arrow). Necrotic cells incorporated the cell-impermeant dye propidium iodide and displayed a pink color when combined with the cyan color of the Hoechst 33342 (Figure 2A, C1, C1a, and C2a, arrowheads). Here, a marked difference in cytotoxicity among the various congeners was observed. All congeners except C2 had 100% toxicity at 24 h of exposure (Figure 2). Conversely, the percentage of cell death with the C2 congener was 3.1 ± 1.3 and 8.9 ± 3.4% for the native mixture. This combination of cell death and inhibition of cell growth resulted in cell density values that were significantly higher for congener C2 when compared with the native compound, with values of 80.3 ± 5.7 and 58.5 ± 7.7% (*P < 0.05) of untreated values, respectively. Both of these values were elevated when compared with those that were obtained for congeners C1, C1a, and C2a, which all were <20% of untreated values (Figure 2B). The data suggest that the C2 component of gentamicin had minimal cytotoxicity and actually reduced the toxicity of the other gentamicin components when present in the native mixture.

Six Days of Daily Exposure to Enantiomer C2 Is Not Nephrotoxic in Sprague-Dawley Rats

Having identified a gentamicin congener with reduced toxicity in cultured cells, we next set out to determine the toxicity of this congener in vivo. Male Sprague-Dawley rats that were given either congener C2 at 100 mg/kg per d or normal saline intraperitoneally for six daily doses showed no elevation in serum creatinine (Figure 3A). In these groups, serum creatinine values remained ≤0.2 mg/dl up to 24 h after the last injection. However, rats that were given the same dose of the native gentamicin compound exhibited an elevation in serum creatinine 24 h after the first injection, with a near tripling of serum creatinine values to a peak value of 0.55 mg/dl 24 h after the fourth injection. We specifically used six daily doses for these studies to allow additional time for the C2 component to induce injury, because it is widely known that serum creatinine rises early in this model when native gentamicin is used. Therefore, even after prolonged exposure to C2, there was no change in serum creatinine values, which remained significantly lower than those of native gentamicin–treated rats (*P ≤ 0.05).

Six Days of Daily Exposure to the C2 Congener Induces Few Alterations in Kidney Morphology

A general histologic survey of the rat kidney tissues was conducted next to determine whether gross morphologic changes accompanied differences that were observed in renal function. Again, these studies were conducted after 7 d of exposure, or six doses of gentamicin. This was to ensure that we would see any delayed nephrotoxicity from the C2 component. These studies occurred at a time when the native gentamicin–treated animals were recovering, as evidenced by a reduction in serum creatinine. Therefore, our studies underestimate the actual histologic damage that was seen early for native gentamicin. H&E-stained kidney sections of rats that were exposed to

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**Figure 2.** Cytotoxicity assays using cultured cells revealed that the first enantiomer separated, which we call congener C2, had markedly reduced toxicity after 24 h of continual exposure. Results in the porcine proximal tubule cell line LLC-PK1 were striking. (A) Apoptosis was assessed using the nuclear dye Hoechst 33342, which labeled nuclei of normal cells uniformly with a lower intensity and the nuclei of apoptotic cells more intensely and demonstrated the presence of condensed apoptotic bodies. The nuclear dye propidium iodide is cell impermeant and labels only necrotic cells, characterized by permeable cell membranes; co-localization of the two dyes gives a reddish-pink color. For all congeners except C2, evidence of widespread necrosis existed (A). For the congener C2, minimal evidence of toxicity was present. A histogram that was generated form the microscopic data revealed a toxicity index for C2 lower than any other individual component and lower than the native compound, 3.1 ± 1.3 and 8.9 ± 3.4%, respectively (B). Cell density for the toxic C1, C1a, and C2a congeners dropped below 20% of control values. The values for C2 were significantly higher than any other congener and also higher than the native compound (*P ≤ 0.05) with reported values of 80.3 ± 5.7 and 58.5 ± 7.7%, respectively. Values are means ± SEM. Bar = approximately 10 μm.
the native gentamicin compound showed extensive proximal tubule cell damage (Figure 3B). Here, proximal tubules from native gentamicin–treated rats contained cast material and shed cells in the lumen (thick arrow). In addition, many of the proximal tubule cells contained large vacuoles that localized around the nucleus (Figure 3B, arrowheads). In contrast, kidney sections from rats that were treated with congener C2 exhibited normal morphology (Figure 3C). Tubular epithelial cells were cuboidal, and the lumens lacked cellular debris. Occasionally, vacuoles appeared within the tubular epithelia in C2-treated rats, but they generally were much smaller in size, fewer in number, and not localized to the perinuclear area
(Figure 3C, arrowheads). Rats that were exposed to normal saline exhibited morphology similar to that seen with the congener C2 (data not shown).

Thin histologic sections also were stained with PAS to localize the glycocalyx that was associated with the brush border membrane of proximal tubules. Here, a violet color that is associated with the glycocalyx-rich microvilli helps delineate changes to the brush border. In rats that were exposed to the native gentamicin compound, the proximal tubular brush border seemed to be reduced in size (Figure 3D, thin arrows). As with the H&E sections, these tubules contained intraluminal debris (Figure 3D, arrows) and intracellular vacuoles (arrowheads). The proximal tubular brush borders of rats that were exposed to congener C2 exhibited more abundant and enriched PAS staining, defining the taller apical membranes (Figure 3E, thin arrows), similar to that observed in saline-treated rats (data not shown). Results from a modified Jablonski score (Figure 3F) to assess damage in the cortex showed that injury was significantly reduced in rats that were treated with C2 as compared with those that were treated with native gentamicin ($P \leq 0.05$).

When tissues from rats that were exposed to the native commercial gentamicin compound or the C2 congener were processed for indirect immunofluorescence localization of gentamicin, a difference in the intracellular accumulation and distribution emerged. The formation of myeloid bodies or cytosegresomes within proximal tubule cells has been a hallmark alteration associated with prolonged exposure to aminoglycosides (14,15). Here, formation of these structures in rats that were exposed to the native gentamicin compound was seen (Figure 3G, arrowheads). In these tissues, the lysosomes appeared swollen and greatly reduced in number, and they often were localized around the nucleus. In contrast, rats that were exposed to congener C2 exhibited what would be deemed normal lysosomal morphology (Figure 3H). In these tissue sections, lysosomes seemed much more numerous, smaller in diameter, and diffusely distributed throughout the cytosol. They were not segregated around the nucleus as noted for the native compound. Tissue sections from rats that were treated with normal saline alone and processed identically for immunofluorescence localization produced no fluorescence associated with gentamicin localization (data not shown).

**Short-Term Exposure to the Individual Congeners Reveals No Difference in Early Nonlysosomal Trafficking**

Previous studies from two laboratories have documented the importance of cytosolic release of aminoglycosides in subsequent LLC-PK1 cell injury (9,16). Therefore, we hypothesized that the non-nephrotoxic C2 component of gentamicin might not be released as rapidly or to the same extent as the other toxic gentamicin components. To test this hypothesis directly, we used our previously reported endosomal/lysosomal quenching and Tyramide signal amplification techniques to evaluate the early phase of cytosolic gentamicin trafficking (9). The results shown in Figure 4 indicate that we could discern no difference in the extent of cytosolic release 1 h after exposure to any gentamicin component. In all cells, for all congeners, there was rapid appearance of a homogeneous cytosolic distribution of gentamicin. Therefore, all components reached the cytosol rapidly, and these data could not explain the differences that were noted in cell injury among the various components.

**Large Perinuclear Accumulations of Cytotoxic Gentamicin Congeners Contain Elements of Both Lysosomes and the Golgi Complex**

To study long-term differences in the intracellular distribution of gentamicin, we undertook additional studies in LLC-PK1 cells. After 24 h of continuous exposure to the native gentamicin mixture, congener C2, or the cytotoxic congeners and a 10,000 molecular weight Texas Red dextran to localize the lysosomes, cells were fixed and processed for the localization of the Golgi complex with a FITC-conjugated lectin from *Helix pomatia* (17) and indirect immunofluorescence localization of gentamicin with a Cy-5–conjugated secondary antibody. The staining patterns that were observed for the native compound, congener C2 and congener C1a (as a representative of the altered morphology seen with all cytotoxic congeners) are shown in Figure 5. Exposure to gentamicin, as either the native mixture or the individual congeners, showed co-localization between the dextran and gentamicin in lysosomes, as expected. In untreated cells or cells that received congener C2, lysosomes seemed numerous, small, and evenly distributed.
throughout the cytosol, as previously noted in the rat studies. In contrast, the lysosomes in cells that were exposed to cytotoxic congeners were located in a perinuclear position and swollen, as seen here with C1a. The Golgi complex in cells that were treated with C2 but not those that were exposed to the cytotoxic congeners was faint. Some staining in these cells also occurred in the periphery of the cell, likely as a result of recognition of carbohydrate moieties at the cell surface. Cells that were exposed to the cytotoxic congeners produced a Golgi staining pattern that was identical to that of lysosomes and gentamicin as seen with C1a. Here, the intensity of the lectin staining was markedly increased with little or no staining within the remaining cytosol or the cell surface. In staining that was reminiscent of immunolocalized native gentamicin in rats, cells that were treated with C1a exhibited large grape-like clusters around the nucleus and contained gentamicin and both lysosomal and Golgi complex markers. Cells that were exposed to C2 lacked these structures, and there was no overlap between the lysosomal and Golgi complex markers. The staining pattern for the native compound was more heterogeneous, with accumulation patterns in between those seen for either C2 or C1a. These data suggest that the cytotoxic gentamicin congeners induce a trafficking abnormality after prolonged exposure. They further indicate that inclusion of the C2 component reduces the intracellular trafficking abnormality that is induced by the nephro-

Figure 5. Long-term exposure to the cytotoxic congeners induced coalescence of lysosomes and the Golgi complex in LLC-PK1 cells. Texas Red dextran (0.5 mg/ml) was given in the media during gentamicin exposure to label the lysosomes. In untreated cells and congener C2-treated cells, the lysosomes appear as small, discrete vesicles found throughout the cellular cytosol. Cells exposed to native gentamicin exhibited some perinuclear accumulation of gentamicin and Texas Red dextran, although both still largely were confined within smaller vesicles. Cells exposed to the cytotoxic congeners, as shown here with C1a, produced much larger swollen vesicles that encompassed the nucleus. When gentamicin was introduced to the cells and visualized, co-localization with the lysosomes occurred. Visualization of the ER-Golgi-intermediate-compartment/cis-Golgi complex with a lectin from Helix pomatia was faint in untreated cells, congener C2–treated cells, and the native gentamicin–treated cells. Movement of the lectin epitope during normal cellular trafficking may account for the faint staining that was seen at this concentration. Cells exposed to congener C1a exhibited intense staining of Helix pomatia, producing a pattern that was identical to that seen with the Texas Red dextran and gentamicin. Bar = approximately 10 μm.
toxic congeners, because treatment with the native gentamicin mixture showed results in between the two extremes.

Discussion

Clinical Implications

Numerous strategies have been developed to minimize the nephrotoxicity that is associated with aminoglycoside antibiotics. Although certain aminoglycoside antibiotics are less nephrotoxic, all still result in an unacceptably elevated level of nephrotoxicity, especially in high-risk patients. Approaches to limit this associated toxicity have included introduction of an agent to alter uptake or intracellular trafficking of aminoglycosides. In this regard, high levels of dietary calcium (18) or the use of polyaspartic acid has shown beneficial effects in animal models (19–22). Another approach has been purification of a nontoxic native component, or modification of the parent compounds to produce less toxic substances. This approach has yielded two commercially available aminoglycosides that have somewhat less nephrotoxicity and commonly are used today. For example, modification of Kanamycin A yielded Amikacin, and Tobramycin was purified from Nebramycin (23).

Early clinical observations indicated the nephrotoxicity of different gentamicin manufacturing lots differed (12). This led Kohlhepp et al. (12) to initiate studies to determine the relative toxicity of the various gentamicin components in animal studies. Unfortunately, at the time of their studies, it was not possible to separate gentamicin cleanly into its various components. Therefore, their studies were conducted using mixtures that were enriched for a given component C1, C1a, or C2. In addition, the C2a component, a component of the native mixture that we showed to have a high level of cellular toxicity, could not be identified in these early studies. Therefore, the actual level of cross-contamination reported in the study may represent an underestimation, because no data as to where and to what extent C2a was present in each of the enriched component mixtures exist.

Using improved technology, we were able to separate gentamicin components into pure fractions. Our data clearly identified the C2 component as nontoxic in cell culture; the consistency between cell lines and the in vivo rat data confirm the nontoxic nature of the C2 component.

In our studies, the non-nephrotoxic C2 congener was found to be just as potent against Gram-positive and multidrug-resistant Gram-negative bacteria as native gentamicin or any of the individual components. Therefore, the C2 congener could have a profound clinical impact in the treatment of infectious diseases. More aggressive and broader therapeutic regimens that now could include patients who previously were deemed unsuitable because of preexisting risk factors may be developed. Moreover, understanding the mechanisms that are inherent to toxicity may provide a means by which effective co-therapies can be developed. Finally, the congener C2 holds great promise as a therapeutic agent for the treatment of genetic diseases that arise from premature stop mutation defects, in which continuous daily doses to increase transcription of complete proteins may be possible (7,8). A series of studies have already shown gentamicin’s potential benefit against diseases such as cystic fibrosis, Hurler’s syndrome, and Duchenne and Becker muscular dystrophies (7,8). Additional data indicate that premature stop codon mutations in the IDUA and p53 genes can be suppressed by gentamicin or Amikacin but not by Tobramycin (24).

Mechanism

Cytosolic release, a pathway for gentamicin trafficking that was identified previously in our laboratory (9) and confirmed recently by Servais et al. (16), was a characteristic shared by all congeners in this study, including the non-nephrotoxic C2 congener. Although this potential explanation for the lack of toxicity that was seen with C2 was eliminated by our studies, a deleterious alteration to the Golgi complex and lysosomes, characterized by coalescing of the two into a large amorphous compartment, was observed. These structures, presumed to be “myeloid bodies” that classically have been associated with gentamicin toxicity (14,15), were produced by the cytotoxic congeners but not with the non-nephrotoxic C2 congener. This alteration was apparent in cultured cells after 24 h of continuous exposure and in proximal tubule epithelial cells in rats that were subjected to daily exposure for 6 d. This effect is similar to the trafficking effects that were induced by Brefeldin A, another fungal metabolite, in certain cell lines (25).

In cells that were exposed to the congener C2, staining with Helix pomatia was faint. This lectin is known to label the cis-Golgi and endoplasmic reticulum–Golgi intermediate compartments. Hence, shuttling of the lectin epitope between the endoplasmic reticulum and Golgi complex could account for the reduced intensity at the concentration used. In contrast, the intensity that was seen at the Golgi complex when the cytotoxic congeners were used (represented by C1a in Figure 5) was very intense and localized in grape-like clusters around the nucleus. We postulate that this arises from an accumulation of Golgi elements in a stagnant organelle. Of note, these alterations occurred within the entire lysosomal pool and not just within newer endosomal bodies that formed after gentamicin exposure, because preincubation with dextran to label lysosomes, followed by a chase and subsequent gentamicin exposure for 24 h, produced identical results (data not shown). Similar structures also were seen in our previous studies and allowed for easy identification of the Golgi complex (9,26). The affects on this pathway were much more severe when cultured cells were exposed to the individual cytotoxic congeners as compared with the native compound. This observation again strongly suggests that the inclusion of the C2 congener, in the native mixture, affords protection against the effects of the cytotoxic congeners. Therefore, on the basis of the data presented here, the naturally occurring variation in the percentage of C2 congener composition of native gentamicin from commercial lot to lot could account for the previous clinical observations regarding variable nephrotoxicity (13).

Conclusion

The gentamicin congener C2 was isolated from native gentamicin and shown to induce no cellular injury and no nephrotoxicity in a rat model of gentamicin toxicity while retaining
normal bactericidal properties. The C2 congener failed to induce the intracellular trafficking abnormalities that were seen with other congeners and native gentamicin. Taken together, these data indicate the C2 congener of gentamicin may be of clinical value for the treatment of serious infections, especially in patients who are at high risk for developing ARF.

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CORRECTION


Please note the following correction in the above article published in the October 2006 issue of *JASN*. In this article, Figure 1A had two of the chromatogram peak labels reversed during the publication process. The original submitted figure is now presented here showing the correct C1 and C1a peaks. This publication error had no effect on the data or its interpretation.