Gentamicin suppresses endotoxin-driven TNF-α production in human and mouse proximal tubule cells

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Zager RA, Johnson AC, Geballe A. Gentamicin suppresses endotoxin-driven TNF-α production in human and mouse proximal tubule cells. Am J Physiol Renal Physiol 293: F1373–F1380, 2007. First published August 15, 2007; doi:10.1152/ajprenal.00333.2007.—Gentamicin is a mainstay in treating gram-negative sepsis. However, it also may potentiate endotoxin (LPS)-driven plasma TNF-α increases. Because gentamicin accumulates in renal tubules, this study addressed whether gentamicin directly alters LPS-driven tubular cell TNF-α production. HK-2 proximal tubular cells were incubated for 18 h with gentamicin (10–2,000 μg/ml). Subsequent LPS-mediated TNF-α increases (at 3 or 24 h; protein/mRNA) were determined. Gentamicin effects on overall protein synthesis ([35S]methionine incorporation), monocyte chemoattractant protein-1 (MCP-1) levels, and LPS-stimulated TNF-α generation by isolated mouse proximal tubules also were assessed. Finally, because gentamicin undergoes partial biliary excretion, its potential influence on gut TNF-α production was probed. Gentamicin caused striking, dose-dependent inhibition of LPS-driven TNF-α production (up to 80% in HK-2 cells/isolated tubules). Surprisingly, this occurred despite increased TNF-α mRNA accumulation. Comparable changes in MCP-1 were observed. These changes were observed at clinically relevant gentamicin concentrations and despite essentially normal overall protein synthetic rates. Streptomycin also suppressed LPS-driven TNF-α increases, suggesting an aminoglycoside drug class effect. Gentamicin doubled basal TNF-α mRNA in cecum and in small intestine after LPS. Gentamicin can suppress LPS-driven TNF-α production in proximal tubule cells, likely by inhibiting its translation. Overall preservation of protein synthesis and comparable MCP-1 suppression suggest a semiselective blockade within the LPS inflammatory mediator cascade. These results, coupled with increases in gut TNF-α/MCP-1 mRNAs, imply that gentamicin may exert protein, countervailing actions on systemic cytokine/chemokine production during gram-negative sepsis.

HK-2 cells; aminoglycosides; acute renal failure; tumor necrosis factor-α

GENTAMICIN TREATMENT of gram-negative sepsis represents a pharmacological paradox: despite its potent bactericidal activity, the drug can act synergistically with endotoxemia to induce or exacerbate acute renal failure (ARF) (37, 38, 44, 45). Multiple mechanisms underlie this interactive form of renal damage and include the following: 1) endotoxin (lipopolysaccharide; LPS) augments renal cortical gentamicin uptake (44), the latter being a critical determinant of the drug’s nephrotoxic effect (5); 2) gentamicin can potentiate evolving ischemic tubular injury pathways (37, 38, 45), such as those activated during endotoxemic renal vasoconstrictive states (20, 27); and 3) both aminoglycosides (34) and LPS can generate reactive oxygen/nitrogen intermediates (14, 33, 35, 36, 46), potentially culminating in severe oxidant renal tubular stress.

Recent studies from this laboratory (40–43) have indicated that diverse forms of acute renal tubular injury (e.g., as induced by myoglobinuria, cisplatin, ischemia, or ureteral obstruction) can enhance renal TNF-α production during endotoxemia. These observations led us to consider an additional hypothesis: that subtle gentamicin nephrotoxicity might, like other forms of renal damage, sensitize the kidney to LPS-driven TNF-α production, a well-defined determinant of evolving ARF (24). To test this hypothesis, we previously injected mice with gentamicin or its vehicle and, 0–24 h later, imposed an intravenous LPS challenge (39). Gentamicin pretreatment approximatively doubled LPS-mediated increases in plasma TNF-α concentrations (39).

Given the fact that gentamicin is devoid of extrarenal cytotoxic effects (excepting the inner ear), we hypothesized that the gentamicin-induced enhancement of LPS-initiated plasma TNF-α increases might be a direct result of increased renal cortical TNF-α production. This was also suggested by augmented renal cortical TNF-α mRNA responses to LPS (39). However, Bennett et al. (3) and Buss et al. (4) convincingly demonstrated that gentamicin can suppress renal tubular cell protein (and potentially cytokine) synthesis. This raises a question as to the direct impact of gentamicin on LPS-mediated tubular TNF-α production. The present study was undertaken to help elucidate this unresolved but clinically relevant issue.

METHODS

Three-Hour LPS Challenge: Gentamicin Effects on HK-2 Cell TNF-α Responses

TNF-α protein levels. Cultured human proximal tubule (HK-2) cells, maintained in keratinocyte serum-free medium (K-SFM), were seeded into T75 flasks, as previously described (26). Routine culture conditions included 100 U/ml penicillin plus 25 μg/ml streptomycin. Approximately 8 h postseeding, the cells were either maintained under control incubation conditions (n = 24 flasks; in K-SFM) or exposed to 500 μg/ml gentamicin (n = 12 flasks; Sigma Chemicals, St. Louis, MO). [The 500 μg/ml dose of gentamicin was chosen because this level is readily achieved in proximal tubular cells in human renal cortex following gentamicin treatment (6, 21) and in human urine during clinical gentamicin treatment (19). This dose is also well within the range used in previous studies of this type (e.g., Ref. 28).] Eighteen hours after gentamicin treatment, one-half of the flasks in the gentamicin pretreated and control groups were either maintained under control conditions for an additional 3 h or challenged with 10 μg/ml Escherichia coli LPS (40, 41) for 3 h. This created the
following four groups (n = 6 flasks each): 1) continuous control incubation; 2) continuous gentamicin treatment; 3) control incubation, followed by the 3-h LPS challenge; and 4) gentamicin treatment, followed by the 3-h LPS challenge.

At the end of this 30 h period, the media were simultaneously removed from each of flasks, the cells were washed with Hank’s buffered salt solution (HBBS), and the cells were recovered by scraping with a rubber policeman. After centrifugation, the protein was extracted and the samples were then assayed for human TNF-α by ELISA (39). Results are expressed as picograms of TNF-α per milligram of cell protein. To document that any contaminating or intracellular gentamicin did not alter the TNF-α assay, three sets of lysates, obtained from LPS-exposed HK-2 cells, were assayed for TNF-α in the presence and absence of added gentamicin (500 μg/ml).

TNF-α mRNA expression. The above protocol was repeated using HK-2 cells cultured in 24 T25 flasks (n = 6 flasks for each of the 4 treatment groups). At the completion of the incubations, total RNA was extracted and assayed for human TNF-α mRNA by RT-PCR (40). The results were factored by simultaneously obtained GAPDH product, used as a housekeeping gene.

Twenty-Four-Hour LPS Challenge: Gentamicin Effects on HK-2 Cell TNF-α Responses

TNF-α protein levels. Sixteen T75 flasks of HK-2 cells were divided into four equal groups, as noted above. However, the cells were harvested 24 h, rather than 3 h, post-LPS treatment. This was done to assess whether a gentamicin-induced alteration in LPS-stimulated TNF-α expression represented a transient (i.e., 3 h) or a more sustainable (e.g., 24 h) event.

TNF-α mRNA expression. The above 24-h protocol was repeated using 12 T25 flasks of HK-2 cells, divided into 4 equal groups. At the completion of the incubations, TNF-α mRNA levels were assessed.

Gentamicin Dose-Response Effect on LPS-Stimulated TNF-α Protein Production

HK-2 cells were treated overnight with the following gentamicin concentrations: 2,000, 200, 100, 50, 25, 10, and 0 μg/ml (n = 4 per group). This was followed by the 3-h LPS challenge, as noted above. Cell TNF-α levels were then assessed. Potential lethal cell injury was gauged by calculating the percent lactate dehydrogenase (LDH) release.

Gentamicin Effects on Cellular TNF-α Release Upon Exposure to LPS

Each of the above experiments assessed LPS/gentamicin effects on intracellular TNF-α levels. The following experiment was undertaken to address whether TNF-α is released from HK-2 cells upon LPS exposure and, if so, whether gentamicin impacts this result. HK-2 cells were grown to near confluence in six T25 flasks in K-SFM as noted above. Six hours postseeding, 500 μg/ml gentamicin was added to the culture medium. Eighteen hours later, three flasks were exposed to the 3-h 10 μg/ml LPS challenge and three flasks remained under control incubations. At the end of the incubations, the cell culture medium was removed and concentrated 10-fold using a 10,000 molecular weight cut-off filter. Cell pellet protein extracts were also prepared. All samples then underwent TNF-α assay.

Gentamicin Effects on LPS-Mediated MCP-1 Induction

MCP-1 protein levels. The following experiment was conducted to ascertain whether gentamicin’s effect on LPS-stimulated TNF-α production was specific for TNF-α or whether other inflammatory mediators might also be involved. To this end, 24 T75 flasks of HK-2 cells were divided into 4 groups: 1) control incubation; 2) 500 μg/ml gentamicin incubation for 18 h; 3) control incubation plus 3-h LPS challenge; and 4) 500 μg/ml gentamicin incubation for 18 h plus 3-h LPS challenge. The cells were then extracted and assayed for human MCP-1 protein (performed by the Cytokine Shared Resource Laboratory, Fred Hutchinson Cancer Research Center, Seattle, WA).

MCP-1 mRNA expression. The above protocol was repeated in 24 T25 flasks of HK-2 cells (4 groups of 6 flasks). After the control/gentamicin incubations with or without the 3-h LPS challenge were completed, HK-2 MCP-1 mRNA was determined by RT-PCR (40) with the results being factored by simultaneously obtained GAPDH product.

Streptomycin Effects on LPS-Driven TNF-α Production

Streptomycin (500 μg/ml) challenge. The following experiment was undertaken to ascertain whether a second aminoglycoside could recapitulate gentamicin’s ability to suppress HK-2 cell cytokine generation. To this end, HK-2 cells, grown either under control conditions or for 18 h with 500 μg/ml streptomycin (Sigma Chemicals), were subjected to the above-described 3-h LPS challenge. Cell extract TNF-α levels were then assessed.

Streptomycin (100 μg/ml) challenge. Streptomycin, in a dose of 100 μg/ml, is frequently used to suppress bacterial growth in cell culture conditions (e.g., Sigma Chemicals). To assess whether this dose of streptomycin could blunt TNF-α responses to LPS, we repeated the above-described streptomycin experiment using a 100 μg/ml streptomycin dose.

Assessments of Protein Synthesis

The following two experiments were undertaken to ascertain whether gentamicin-induced reductions in LPS-stimulated TNF-α production (see RESULTS) was simply a reflection of a generalized suppression of total HK-2 cell protein synthesis. To this end, HK-2 cell protein synthesis was assessed using two independent methods: 1) [35S]methionine incorporation and 2) production of total cell lactate dehydrogenase (a surrogate marker of total HK-2 cell protein production; Ref. 15).

[35S]methionine incorporation as an assessment of protein synthesis. HK-2 cells were cultured in six-well Costar plates either under control conditions or in the presence of 500 μg/ml gentamicin. Approximately 18 h later, [35S]methionine was added to each well (50 μCi/ml; Perkin Elmer Life and Analytical Sciences) with or without 10 μg/ml LPS. Three hours later, cell lysates were prepared by removing the medium, washing the monolayers with PBS (4°C), and lysing the cells in 2% SDS (65°C). After a brief sonication to reduce viscosity, the protein concentrations were determined by o-phenylenediamine assay (8). Trichloroacetic acid precipitable counts were measured (11).

Cellular LDH levels. Four 24-well Costar plates were seeded with HK-2 cells. Approximately 8 h later, 0, 500, or 2,000 μg/ml gentamicin were added. Twenty-four hours later, the cells were detached and total LDH content per well was determined (15). The percentage of cellular LDH release was measured to assess whether any lethal cell injury had occurred.

Gentamicin Effects on TNF-α Production by Mouse Isolated Proximal Tubules

The following experiment was conducted to determine whether gentamicin’s effect on LPS-driven TNF-α production in HK-2 cells was HK-2 cell specific or whether it could be expressed in other cells of proximal tubular cell origin. To this end, six male CD-1 mice (25–30 g; Charles River Laboratories, Wilmington, MA) were injected with 40 mg/kg gentamicin. The following morning, a second dose of gentamicin was administered. Six control mice received sham injection. One hour after the second injection, the mice were deeply anesthetized with pentobarbital (50 mg/kg ip), the kidneys were dissected through a midline abdominal incision, the renal cortices were dissected, and proximal tubule segments were isolated.
as previously described (43). They were incubated at 37°C in experiment buffer (43) under oxygenated conditions (95% O2-5% CO2) for 45 min in the presence or absence of 100 μg/ml LPS and in the presence or absence of 2 mM glycine (the latter was added to minimize tubule injury). At the end of the incubations, tubule buffer samples were assayed for TNF-α by ELISA, as noted above. The percent increases in buffer TNF-α (end of vs. start of incubations) were assessed and compared between the control and gentamicin-pretreated groups. Percent LDH release was determined to assess possible lethal cell damage. This protocol (and that described immediately below) were approved by the Fred Hutchinson Center Animal Institute Care and Use Committee.

Cecal and Small Intestinal TNF-α/MCP-1 mRNA Responses to Gentamicin ± LPS

It was previously noted that gentamicin treatment increases hepatic TNF-α mRNA and TNF-α mRNA responsiveness to LPS (39). Because gentamicin undergoes a small amount of biliary excretion into gut (5, 25, 29), the impact of gentamicin on small intestinal and cecal TNF-α mRNA expression was assessed. Ten mice were injected intraperitoneally with 40 mg/kg gentamicin or an equal volume of saline. A second dose of gentamicin or saline was administered approximately 18 h later. One hour after this second injection, the mice were deeply anesthetized with pentobarbital, a midline laparotomy was performed, and pieces of small intestine and cecum were resected. The samples were iced, intraluminal contents were manually expunged, and RNA was extracted and assayed for TNF-α mRNA by RT-PCR (40). Results were expressed as a ratio to simultaneously obtained GAPDH product. As a comparison, MCP-1 mRNA (40) levels were also determined.

To assess postgentamicin LPS responsiveness, the above experiment was repeated, but 1 h after the second gentamicin or saline injection, 2 mg/kg LPS were injected via the tail vein. Two hours later, small intestinal and cecal TNF-α-, MCP-1-, and GAPDH mRNA expression was determined.

Calculations and Statistics

All results are means ± SE. TNF-α/MCP-1 concentrations are expressed as picograms per milligram of cell protein extract or as picograms per milliliter of culture medium. Statistical comparisons were performed using unpaired or paired Student’s t-test. Significance was judged at P < 0.05.

RESULTS

Three-Hour LPS Challenge: Gentamicin Effects on HK-2 Cell TNF-α Expression

TNF-α protein levels. Essentially no TNF-α could be detected in either control cells or cells exposed overnight to 500 μg/ml gentamicin. After a 3-h LPS exposure, marked TNF-α protein increases were observed (see Fig. 1, top). Gentamicin treatment suppressed these LPS-driven TNF-α increases by ~75%. Gentamicin did not alter the performance of the TNF-α assay: essentially identical TNF-α levels were observed in cell lysates obtained from LPS-challenged HK-2 cells assayed with or without subsequent gentamicin (500 μg/ml) addition (70 ± 4 vs. 68 ± 3 pg/mg protein).

TNF-α mRNA expression. Overnight gentamicin treatment did not independently alter TNF-α mRNA. The 3-h LPS challenge induced an approximately eightfold increase in TNF-α mRNA compared with control levels (Fig. 1, bottom). Overnight gentamicin treatment modestly, but significantly, enhanced these LPS-driven TNF-α mRNA increases.
recapitulated the results seen with the above-described 3-h LPS challenge.

**Gentamicin Dose-Response Effect on LPS-Stimulated TNF-α Protein Production**

Figure 2 presents the degree to which differing doses of gentamicin suppressed HK-2 cell TNF-α increases in response to the 3-h LPS challenge. The results are presented as percent suppression compared with simultaneously obtained values in 3-h LPS challenged/non-gentamicin-treated control cells. A steep inverse dose-response relationship was observed, reaching a maximum of ~80% suppression at 2,000 μg/ml. Even at the 10 or 25 μg/ml gentamicin dosage, a significant suppression of TNF-α production was observed ($P < 0.025$ for each).

**Extracellular vs. Intracellular TNF-α Levels After Exposure to LPS**

Cell culture medium obtained from HK-2 cells in the absence of LPS contained no detectable TNF-α. After a 3-h LPS exposure, TNF-α was detected in the cell culture medium (77 ± 3 pg/ml; Fig. 3, top). Gentamicin pretreatment blunted this LPS response by ~67% (the values in Fig. 3 are divided by 10 to correct for the 10-fold medium concentration before assay). This 67% blunting of LPS-mediated extracellular TNF-α increase was matched by a 60% reduction in intracellular TNF-α levels (Fig. 3, bottom).

**Gentamicin Effects on LPS-Mediated MCP-1 Induction**

**MCP-1 protein levels.** MCP-1 was detectable in HK-2 cells when grown under normal culture conditions (~35 pg/mg protein; Fig. 4, top). Gentamicin (500 μg/ml) suppressed this basal expression by ~40% (to ~20 pg/mg protein). The 3-h LPS challenge caused an acute eightfold increase in MCP-1 levels over basal values (to ~300 pg/mg). When the LPS challenge was conducted in gentamicin-conditioned cells, an ~40% suppression in MCP-1 accumulation was observed (Fig. 4, top).

**MCP-1 mRNA expression.** As shown in Fig. 4, bottom, gentamicin had no significant impact on MCP-1 mRNA levels in the absence of LPS. MCP-1 mRNA values rose approximately eightfold in response to LPS (quantitatively mirroring the above-noted MCP-1 protein increases). Gentamicin modestly, but significantly ($P < 0.035$), enhanced the LPS-induced MCP-1 mRNA increases. Thus these MCP-1 protein and mRNA results completely paralleled those from the above-described TNF-α experiments (~40–50% suppression of LPS-mediated increases in TNF-α/MCP-1 protein despite an augmentation of LPS-induced increases in TNF-α/MCP-1 mRNAs).
Streptomycin Effects on LPS-Driven TNF-α Production

Streptomycin at a dose of 500 µg/ml caused an approximate 30% reduction in LPS-driven TNF-α production (80 ± 6 vs. 57 ± 4 pg/ml; P < 0.05). Even at a dose of 100 µg/ml, streptomycin significantly (17%) reduced LPS-driven TNF-α generation (P < 0.015).

HK-2 Cell Protein Synthesis

Over a 24-h period, a slight, dose-dependent decrease in total cell LDH content (a surrogate marker of HK-2 cell protein synthesis; Ref. 15) was detected (5 and 10% reductions with 500 and 2,000 µg/ml gentamicin dosages, respectively; P < 0.01–0.03; Fig. 5, top). Neither gentamicin nor LPS caused any lethal HK-2 cell injury, assessed as percent LDH release (gentamicin alone, LPS alone, and gentamicin + LPS: all 8 ± 1% LDH release; also consistent with results from Ref. 28).

During a 3-h pulse, no significant difference in [35S]methionine incorporation into protein was observed in control compared with gentamicin-treated cells. LPS addition did not alter [35S]methionine incorporation. Gentamicin did not alter [35S]methionine incorporation during this challenge with LPS (Fig. 5, bottom).

Fig. 4. Gentamicin effects on HK-1 cell monocyte chemoattractant protein-1 (MCP-1) generation in response to LPS. In contrast to TNF-α, MCP-1 could be detected in HK-2 cells under basal (non-LPS stimulated; Cont) conditions (top). Gentamicin treatment suppressed these basal MCP-1 levels by ~50% (top). MCP-1 levels rose ~8-fold in response to LPS, and gentamicin suppressed this LPS-stimulated MCP-1 response by ~50% (top). Gentamicin did not independently alter MCP-1 mRNA levels (bottom). Both control and gentamicin-treated cells mounted dramatic increases in MCP-1 mRNA in response to LPS (bottom). This increase was slightly, but significantly (P < 0.035), greater in the gentamicin treatment group. NS, not significantly different.

Fig. 5. Gentamicin effects on protein synthesis. Total lactate dehydrogenase (LDH) levels (a surrogate marker for HK-2 cell protein synthesis; Ref. 15) were assessed in cells after 24-h exposures to 0, 500, or 2,000 µg/ml gentamicin. Approximately 5 and 10% reductions were observed with 500 and 2,000 µg/ml gentamicin. Approximately 5 and 10% reductions were observed with 500 and 2,000 µg/ml gentamicin dosages, respectively, compared with values in control cells (top). [35S]methionine protein incorporation did not significantly differ between control cells and 18-h gentamicin-treated cells (bottom). Gentamicin also did not reduce [35S]methionine incorporation during this challenge with LPS.
Gentamicin Effects on TNF-α Production by Mouse Isolated Proximal Tubules

Incubation of isolated tubules from gentamicin and control mice revealed identical percent LDH releases (9 ± 1%) after completion of the 45-min incubations, whether or not glycine was present. No difference in baseline TNF-α concentrations was apparent between tubules harvested from gentamicin and control mice (44 ± 7 vs. 45 ± 16 pg/mg tubule protein). Incubating control tubules with LPS caused an 80 ± 16% increase over these baseline TNF-α levels. Conversely, only a 29 ± 14% increase in TNF-α levels was seen in tubules harvested from gentamicin-pretreated animals (P = 0.026 vs. control tubules). Coincubation with glycine did not alter these results.

Cecal and Small Intestinal TNF-α/MCP-1 mRNA Responses to Gentamicin ± LPS

In the absence of LPS, gentamicin treatment doubled cecal TNF-α and MCP-1 mRNA levels (Fig. 6, top). Conversely, it failed to alter either mRNA level in small intestine (not shown).

LPS evoked ~10-fold increases in both small intestinal and cecal TNF-α and MCP-1 mRNAs (vs. control tissue levels, as shown in Fig. 6, top; P < 0.001). Gentamicin-treated mice manifested twice the amount of LPS-triggered TNF-α mRNA increases in small intestine compared with LPS-treated controls (Fig. 6, bottom). However, gentamicin did not alter intestinal TNF-α mRNA LPS responses (Fig. 6, bottom). Although gentamicin increased both TNF-α and MCP-1 mRNA levels in the absence of LPS (Fig. 6, top), it did not alter their responsiveness to LPS injection.

DISCUSSION

Following systemic administration, gentamicin is transported into proximal tubular cells (5, 9, 23). Although clinical therapeutic peak and trough plasma gentamicin levels are generally within the range of 2–30 μg/ml (depending on dosage and three times a day vs. daily administration intervals; Ref. 5), intrarenal concentrations of 200–1,000 μg/ml are readily attained (6, 21). The results of the present study indicate that gentamicin levels within this clinically relevant range can profoundly impact proximal tubular cell TNF-α responses to LPS. For example, when HK-2 cells were exposed to 500 μg/ml (a dose chosen to simulate toxic cortical gentamicin levels), ~50% reductions in LPS-driven TNF-α production resulted. Dose-titration studies revealed a steep inverse relationship (up to 80% TNF-α suppression). Even at 10–25 μg/ml (simulating peak therapeutic plasma gentamicin concentrations), a statistically significant blunting of LPS-stimulated TNF-α responses was observed. A relevant question is whether these results, obtained with HK-2 cells, are specific for this particular cell line or whether they might have more broad-based relevance. The isolated tubule studies indicate that the latter is likely the case: proximal tubules harvested from gentamicin-treated mice manifested an ~75% suppression of in vitro TNF-α responses to LPS compared with control tubules. Thus at least two proximal tubular cell preparations, one from mice and one derived from humans, manifested comparable blunting of LPS-driven TNF-α increments.

To ascertain whether the above results were gentamicin specific or whether they could be recapitulated by a second aminoglycoside, LPS-driven TNF-α responses in HK-2 cells were assessed in the presence of streptomycin. Again, a suppression of LPS-driven TNF-α production was observed. This finding indicates that the above-described gentamicin results likely reflected a drug class, rather than a drug-specific, action. Particularly noteworthy was the finding at a concentration of ~100 μg/ml that both drugs suppressed LPS-driven TNF-α increases. Many, if not most, laboratories add aminoglycosides to cells in culture as prophylaxis against bacterial overgrowth. The finding that gentamicin and streptomycin each suppressed LPS-driven TNF-α production, and at concentrations commonly employed in cell culture experiments (e.g., Sigma Laboratories), suggests a potentially important technical consideration for investigators, particularly when undertaking studies of LPS-initiated inflammatory signaling.

Aminoglycosides are well known to inhibit protein synthesis via ribosomal binding with translation inhibition. Mistransla-
tion of mRNAs, possible changes in stop codon function, and inhibition of DNA replication also may be involved (1–4, 13, 18, 22, 30). The relevance of these observations to kidney is illustrated by studies from Bennett et al. (3) and Buss et al. (4). These investigators documented gentamicin-induced suppression of protein synthesis in both whole renal cortex and renal microsomal preparations. These observations provide a seemingly straightforward explanation for the current results: that the observed suppression of LPS-initiated TNF-α production simply reflected a generalized suppression of protein synthesis. However, additional studies indicated that this is clearly not the case. First, 18-h exposures to either 500 or 2,000 μg/ml gentamicin caused only a 5–10% decrease in total cell LDH content, a surrogate marker of HK-2 cell protein synthesis (15).

Second, when protein synthesis was directly assessed during a 3-h [35S]methionine pulse, no significant reduction was produced by 500 μg/ml gentamicin exposure. Third, when gentamicin-treated and control HK-2 cells were challenged with LPS, no significant difference in [35S]methionine incorporation was observed despite a corresponding 50% reduction in TNF-α levels. Thus these data imply that gentamicin exerts a preferential TNF-α suppressive effect.

To explore whether this result was TNF-α specific or whether other inflammatory mediators might be involved, we assessed MCP-1 levels in control and LPS-challenged HK-2 cells with or without 500 μg/ml gentamicin exposure. Unlike TNF-α, which was below the level of detection under nonstimulated conditions, MCP-1 could be measured in the absence of LPS. Gentamicin suppressed these basal MCP-1 values by ~50%. When the cells were challenged for 3 h with LPS, MCP-1 levels rose eightfold over basal values. Gentamicin also dampened these LPS-mediated MCP-1 increases, again by ~50%. In composite, these results suggest that gentamicin may suppress a number of inflammatory mediators within the LPS signaling cascade.

Although gentamicin can inhibit ribosomal protein synthesis, an alternative explanation for blunted TNF-α/MCP-1 generation with LPS exposure could potentially be a failure of TNF-α/MCP-1 gene transcription. To explore this possibility, we measured TNF-α and MCP-1 mRNA levels in gentamicin-treated HK-2 cells with or without LPS exposure. Under basal conditions, gentamicin failed to alter either mRNA level. However, it seemingly augmented LPS-stimulated TNF-α/MCP-1 mRNA increases. Of note, these mRNA results exactly parallel those that we previously observed in intact renal cortex (where gentamicin loading also enhanced LPS-initiated TNF-α/MCP-1 mRNA induction; Ref. 39). Thus decreased translation, rather than suppressed transcription, appears to be the more likely explanation for the gentamicin-induced TNF-α/MCP-1 hyporesponsive state.

The finding of suppressed tubular cell TNF-α production during LPS stimulation appears paradoxical, given our previous observation that gentamicin augmented LPS-induced increases in circulating TNF-α levels (39). One potential explanation for this paradox could be that gentamicin stimulates cellular TNF-α release. If so, this process could explain both low renal tubular cell and elevated circulating TNF-α levels. However, this theoretical possibility seemingly has been excluded, because extracellular and intracellular TNF-α levels were equally suppressed by gentamicin treatment (Fig. 5). An alternative hypothesis is that gentamicin might stimulate TNF-α generation at extrarenal sites and that the latter (rather than tubular cells) are responsible for heightened circulating TNF-α levels. Because gentamicin undergoes partial biliary excretion and can achieve intestinal concentrations approaching 40% of peak plasma levels (24, 29), we tested its impact on gut TNF-α and MCP-1 mRNA levels. In the absence of LPS, gentamicin doubled TNF-α/MCP-1 mRNA expression in cecum. It also doubled small intestinal TNF-α mRNA responses to LPS. The explanation for these intestinal/colonic actions remains highly speculative at this time. However, three points are notable in this regard. First, aminoglycosides can exert plasma membrane actions, e.g., at sites adjacent to LPS binding sites (7, 10, 16). This could potentially alter LPS activity without requiring cellular gentamicin uptake (which is largely renal specific). Second, aminoglycosides can release LPS from colonizing intestinal bacteria (12, 17, 31, 32). Once released, endogenous LPS might initiate intestinal wall TNF-α mRNA production and/or sensitize (41) to subsequent LPS exposure. Third, our laboratory previously reported that gentamicin treatment elevates hepatic TNF-α mRNA (39). This further supports the concept that gentamicin can impact TNF-α expression at extrarenal sites. In light of these three considerations, gentamicin-induced reductions in renal tubular cell TNF-α protein translation and increases in circulating TNF-α levels are not necessarily inconsistent. Finally, it is notable that in the current studies, gentamicin failed to induce any lethal cell injury, as assessed by LDH release. Thus it is possible that with the induction of in vivo gentamicin nephrotoxicity, secondary “downstream” mechanisms are activated (such as those previously noted; Refs. 24, 40–43) that stimulate renal cytotoxic production.

In conclusion, this study demonstrates that gentamicin can directly inhibit LPS-mediated TNF-α generation in proximal tubular cells. The finding that MCP-1 generation is also suppressed but that overall protein synthesis is well maintained implies a preferential blockade of selected products within the LPS signaling cascade. Preservation or enhancement of LPS-stimulated HK-2 cell TNF-α/MCP-1 mRNA expression suggests that reductions in TNF-α/MCP-1 translation are likely involved. These changes can be observed at readily attainable plasma and renal cortical concentrations and with dosages that are used in cell culture experiments. This suggests potential clinical and experimental relevance. Finally, that gentamicin can alter hepatointestinal TNF-α/MCP-1 mRNAs raises the possibility that aminoglycosides may exert protein actions during gram-negative sepsis that extend well beyond their traditionally recognized bactericidal and nephrotoxic effects.

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REFERENCES


