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Prostacyclin protects renal tubular cells from gentamicin-induced apoptosis via a PPARα-dependent pathway

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To study the protective effect of prostacyclin (PGI2) we increased PGI2 production by infected NRK-52E cells with an adenovirus carrying cyclooxygenase-1 and prostacyclin synthase. PGI2 overexpression protected these cells from gentamicin-induced apoptosis by reducing cleaved caspase-3 and caspase-9, cytochrome *c*, and decreasing generation of reactive oxygen species. Expression of the nuclear receptor of PGI2, peroxisome proliferator-activated receptor-α (PPARα), was reduced during gentamicin treatment of the cells, while its overexpression significantly inhibited gentamicin-induced apoptosis and the amount of cleaved caspase-3. Transformation with PPARα short interfering RNA abolished the protective effect of PGI2 overproduction in gentamicin-treated cells. The PPARα activator docosahexaenoic acid given to gentamicin-treated mice significantly reduced the number of apoptotic cells in renal cortex, but this protective effect was not seen in PPARα knockout mice. Our study suggests that increased endogenous PGI2 production protects renal tubular cells from gentamicin-induced apoptosis through a PPARα-signaling pathway.

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Gentamicin is a widely used aminoglycoside antibiotic for the treating Gram-negative bacterial infection, but its clinical use is limited because gentamicin-induced acute renal failure with acute tubular necrosis occurs in about 20% of patients.[1] Although the intravenously administered gentamicin is almost entirely eliminated by the kidney, a small but toxic portion is selectively reabsorbed and accumulated in the proximal tubular cells.[1,2] Inducing apoptosis is an important cytotoxic mechanism of gentamicin, which has been reported in gentamicin-treated renal proximal tubular cells and mesangial cells.[3–5] Servais and co-workers[4,6] reported that the concentration of gentamicin in the 1- to 3-mM range is related to the onset of gentamicin-induced apoptosis. The mitochondrial pathway has been reported to involve in gentamicin-induced apoptosis in LLC-PK1 cells,[6] which induces a major caspase activation to cause apoptosis in mammalian cells.[7] Additionally, reactive oxygen species (ROS) are often responsible for the mitochondria-mediated signaling pathway of apoptosis. A lot of *in vivo* and *in vitro* evidence indicates that ROS are important mediators of gentamicin-induced apoptosis.[8] Therefore, the ROS-mediated apoptosis signaling plays a major role in gentamicin-induced cytotoxicity.

Prostacyclin (PGI_2), one of the major prostaglandins (PGs), is originated from arachidonic acid by the cyclooxygenase (COX) system coupled to the action of PGI_2 synthase (PGIS).[9] PGI_2 acts on platelets and blood vessels through its specific cell-surface receptor (IP receptor), thereby inhibiting platelet function and dilating blood vessels.[10] Besides, recent reports show that PGs are also the ligands of peroxisome proliferator-activated receptors (PPARs), which belong to a family of ligand-activated transcription factors.[11] The agonists of PGI_2, cPGI and iloprost, can effectively induce DNA binding and transcriptional activation by PPARα and PPARδ,[12] but the same effect did not exist in those experimental conditions with PGI_2 treatment alone, possibly because the chemical instability of this PG precluded it to reach the nuclear target. PGI_2 is also known to inhibit leukocyte functions such as migration and ROS production[13]

and inhibit mesangial cell proliferation.[14] Another PGI_2 analogue, beraprost, has been reported to prevent radiocontrast nephropathy in LLC-PK1 cells.[15] These data reveal the protective effect of PGI_2 on certain cell types.

Recently, we selectively augmented PGI_2 production through adenovirus-mediated transfer of genes for COX-1 and PGIS to rat renal tubular cells, and protected cells from the apoptosis induced by adriamycin, an antitumor anthracycline antibiotic.[16] This implies the therapeutic potential of endogenous PGI_2 for nephrosis. Moreover, recent studies have established that the use of fibrates, PPARα ligands, ameliorates ischemia–reperfusion and cisplatin-mediated proximal tubule cell death by prevention of lipotoxicity, inhibition of fatty acid oxidation, and prevention of renal inflammation.[17–20] Therefore, we hypothesize that PPARα is involved in the protective mechanism of PGI_2 because this PG is a potential activator for PPARα. In this study, we intended to evaluate the protective effect of PGI_2 on gentamicin-induced injury in rat renal tubular cells with the adenovirus-mediated bicistronic COX-1/PGIS transfection, and to investigate PPARα's *in vitro* and *in vivo* protective effects.

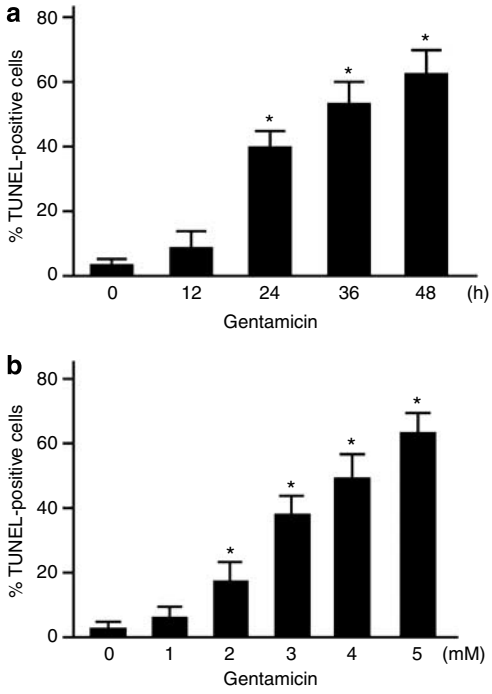

Figure 1 | Gentamicin-induced apoptosis in NRK-52E cells. (**a**) Time-dependent apoptosis induced by gentamicin as revealed by TUNEL assays. NRK-52E cells were treated with 3 mM of gentamicin from 12 to 48 h, harvested, stained with TUNEL assay, and examined by fluorescence microscopy. The level of apoptosis was presented as the percentage of TUNEL-positive cells for each treatment. (**b**) Dose-dependent apoptosis induced by gentamicin as revealed by TUNEL assays. NRK-52E cells were treated with gentamicin at 1–5 mM for 24 h. Results are the mean ± s.d. ($n = 6$). *$P < 0.05$ compared with the group without gentamicin treatment.

RESULTS

Ad-COX-1/PGIS transfection protects NRK-52E cells against gentamicin-induced apoptotic injury

To determine the gentamicin-induced apoptosis in rat renal tubular cell NRK-52E, we treated NRK-52E cells with gentamicin and detected them with enzymatic labeling of DNA strand breaks using terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling (TUNEL). With different administration time, the apoptosis cells were markedly increased by treatment with 3 mM of gentamicin for 24 h, and ascended along the administration time (Figure 1a). In the dosage test, 2 mM of gentamicin was a minimal requirement to induce significant apoptosis in NRK-52E cells within 24 h (Figure 1b).

The production of PGI_2 was typically monitored by measurement of 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) because 6-keto-$PGF_{1\alpha}$ is a stable product of the non-enzymatic hydration of PGI_2. Compared with the adenoviral-human phosphoglycerate kinase (Ad-HPGK) control in Figure 2a, Ad-COX-1/PGIS transfection increased PGI_2 levels in a dose-dependent manner. We also examined the protective effect of Ad-COX-1/PGIS with TUNEL staining. Figure 2b shows that Ad-COX-1/PGIS transfection reduced gentamicin-induced apoptosis in a dose-dependent manner. The result of the study also reveals that the endogenous PGI_2 increase caused by Ad-COX-1/PGIS transfection protects rat renal tubular cells from gentamicin-induced apoptosis.

Effect of Ad-COX-1/PGIS transfection on apoptosis signaling induced by gentamicin in NRK-52E cells

To evaluate the mechanism of the protective effect of Ad-COX-1/PGIS transfection on gentamicin-induced apoptosis, the influence of Ad-COX-1/PGIS transfection on the cellular uptake of gentamicin was first monitored. NRK-52E cells were transfected with Ad-COX-1/PGIS or Ad-HPGK at 40 multiplicity of infection (MOI) for 2 days, and then treated with 3 mM of gentamicin. As shown in Figure 3a, there was a basal level of gentamicin in the 0-h groups. This may result from gentamicin adhering to the cell membranes of NRK-52E cells. After gentamicin treatment, the concentration of cytosol gentamicin reached a maximum within 30 min. Compared with Ad-HPGK transfection, Ad-COX-1/PGIS transfection did not influence the concentration of cytosol gentamicin. This result reveals that Ad-COX-1/PGIS transfection did not influence the cellular uptake of gentamicin. We next examined whether Ad-COX-1/PGIS transfection prevents gentamicin-induced ROS formation because ROS generation is involved in gentamicin-induced apoptosis. Ad-COX-1/PGIS- or Ad-HPGK-transfected cells were treated with 3 mM of gentamicin for 24 h. Gentamicin-induced increases in intracellular ROS were revealed by fluorescent intensities of 2′,7′-dichlorofluorescein (DCF). As shown in Figure 3b, Ad-COX-1/PGIS transfection significantly inhibited gentamicin-induced ROS formation. On the other hand, Ad-COX-1/PGIS transfection significantly induced the activity of antioxidant enzymes, superoxide dismutase (SOD) and catalase, in NRK-52E cells (Figure 3c and d).