Pyruvate dehydrogenase kinase 4 deficiency attenuates cisplatin-induced acute kidney injury

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Clinical prescription of cisplatin, one of the most widely used chemotherapeutic agents, is limited by its side effects, particularly tubular injury–associated nephrotoxicity. Since details of the underlying mechanisms are not fully understood, we investigated the role of pyruvate dehydrogenase kinase (PDK) in cisplatin-induced acute kidney injury. Among the PDK isoforms, PDK4 mRNA and protein levels were markedly increased in the kidneys of mice treated with cisplatin, and c-Jun N-terminal kinase activation was involved in cisplatin-induced renal PDK4 expression. Treatment with the PDK inhibitor sodium dichloroacetate (DCA) or genetic knockout of PDK4 attenuated the signs of cisplatin-induced acute kidney injury, including apoptotic morphology of the kidney tubules along with numbers of TUNEL-positive cells, cleaved caspase-3, and renal tubular injury markers. Cisplatin-induced suppression of the mitochondrial membrane potential, oxygen consumption rate, expression of electron transport chain components, cytochrome c oxidase activity, and disruption of mitochondrial morphology were noticeably improved in the kidneys of DCA-treated or PDK4 knockout mice. Additionally, levels of the oxidative stress marker 4-hydroxynonenal and mitochondrial reactive oxygen species were attenuated, whereas superoxide dismutase 2 and catalase expression and glutathione synthetase and glutathione levels were recovered in DCA-treated or PDK4 knockout mice. Interestingly, lipid accumulation was considerably attenuated in DCA-treated or PDK4 knockout mice via recovered expression of peroxisome proliferator-activated receptor-α and coactivator PGC-1α, which was accompanied by recovery of mitochondrial biogenesis. Thus, PDK4 mediates cisplatin-induced acute kidney injury, suggesting that PDK4 might be a therapeutic target for attenuating cisplatin-induced acute kidney injury.

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Cisplatin is one of the most widely used chemotherapeutic agents for the treatment of various types of solid tumors in the lungs, ovary, testis, and other organs.[1,2] However, the use of cisplatin is limited by its side effects, particularly tubular injury–associated nephrotoxicity.[1–3] Although many studies have suggested potential mechanisms that may be involved in cisplatin-induced kidney injury,[3–17] the details are not yet fully understood.

Previous studies suggest that cisplatin induces tubular cell damage and death through multiple signaling pathways and factors.[3–17] Among these, mitochondria are an important target organelle in cisplatin-induced kidney injury.[3,10,11,13,17] Because the interior of mitochondria is negatively charged, positively charged cisplatin metabolites can easily cross-link mitochondrial DNA and inhibit its synthesis in kidney tubular cells.[2,13] Cisplatin also reduces the mitochondrial membrane potential by reducing the protein levels of the electron transport chain and increasing the levels of reactive oxygen species (ROS), leading to kidney tubule cell death.[3,10,11,13] In addition, cisplatin induces tubular cell damage and death through the accumulation of toxic fatty acids, which occurs via direct inhibition of the binding of

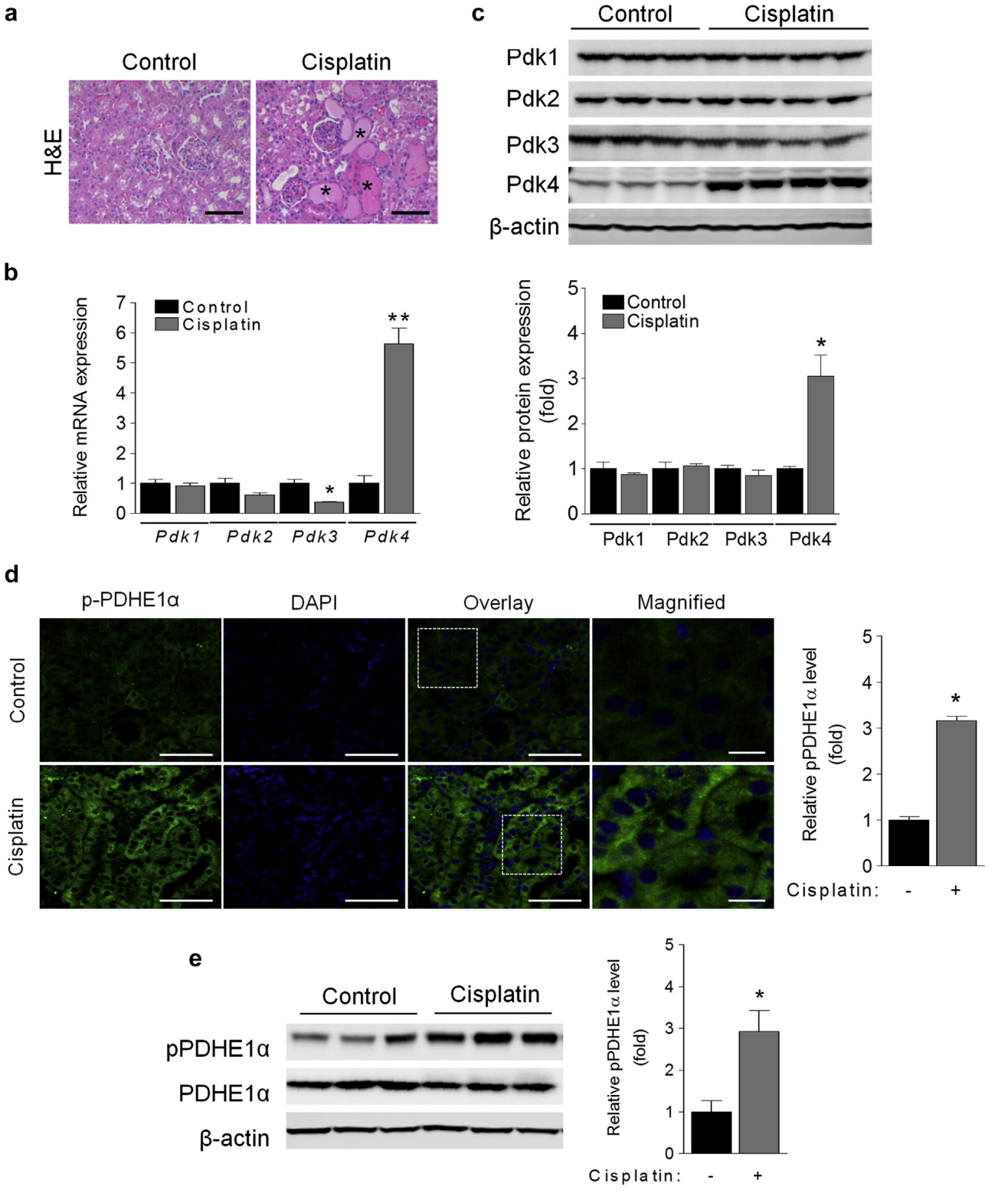

Figure 1 | Pyruvate dehydrogenase kinase (PDK)4 is upregulated in the kidneys of cisplatin-treated mice. (**a**) Hematoxylin and eosin (H&E) staining of mouse kidneys (original magnification ×400; bar = 250 μm, *damaged tubules). Eight-week-old C57BL6 mice were treated with cisplatin (20 mg/kg) and killed after 3 days. (**b**) Renal PDK mRNA expression in mice measured by quantitative reverse-transcriptase polymerase chain reaction. Data are the mean ± SEM ($n = 5$). *$P < 0.01$ versus control (PDK3); **$P < 0.05$ versus control (PDK4). (**c**) Western blot analysis of renal PDK protein expression. Expression of each PDK isoform was quantified (right panel). Data are the mean ± SEM ($n = 5$). *$P < 0.01$ versus control (PDK4). (**d**) Immunofluorescence staining of renal pPDHE1α expression. (Original magnification ×200; bar = 250 μm; bar in the magnified images = 50 μm. Green: p-PDHE1α, blue: 4′,6-diamidino-2-phenylindole [DAPI].) The graph shows the quantitative analysis of pPDHE1α levels. Data are the mean ± SEM of 5 random fields from each kidney. *$P < 0.01$ versus cisplatin (−). (**e**) Western blot analysis of PDHE1α phosphorylation in kidneys. Graphs showing the results of quantitative analysis are shown on the right. Data are the mean ± SEM ($n = 5$). *$P < 0.01$ versus control.