Protective Action of Prostamide E2 on Homocysteine-induced NLRP3 Inflammasome Activation and Podocyte Injury

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ABSTRACT

Recent studies have demonstrated that homocysteine (Hcy)-induced podocyte damage and subsequent podocyte injury and glomerular sclerosis are attributed to the activation of NLRP3 inflammasomes. Given the possible immunosuppressive effect of endogenous cannabinoids, the present study was designed to test whether prostamide E2 (PE2), COX-2 metabolite of anandamide (AEA) diminishes Hcy-induced NLRP3 inflammasome activation and podocyte injury. By confocal microscopy, Hcy treatment of podocytes was found to induce NLRP3 inflammasome formation as demonstrated by the increase in colocalization between three major inflammasome proteins—NLRP3, ASC and caspase-1. Accompanying these effects, elevated Hcy resulted in increased caspase-1 activity and IL-1β production in podocytes. This increase in Hcy-induced NLRP3 inflammasome formation and activation was substantially inhibited by PE2 pretreatment with a maximum inhibition of 94% of the Hcy-induced caspase-1 activity and IL-1β production. Anandamide only had such inhibitory effect on inflammasome activation at a concentration 10 times higher than PE2, which was blockable by COX-2 inhibitor. Furthermore, PE2 was found to preserve podocyte morphology by maintaining the distinct arrangement of F-actin fibers normally lost in response to Hcy. It also prevented podocyte dysfunction by restoring Hcy-induced suppression of VEGF production and secretion and by inhibition of Hcy-induced decreases in podocin and increases in desmin. Together, these results provide evidence that PE2 may exert its anti-inflammatory action by suppression of Hcy-induced NLRP3 inflammasome activation and prevention of podocyte injury and dysfunction (supported by NIH grants HL07244, HL-75316 and DK4927).

BACKGROUND

- Our previous studies have demonstrated that Hcy-induced NLRP3 inflammasomes activation is one of major initial mechanism resulting in podocyte injury.
- Cyclooxygenase-2 (COX-2) and its major product Prostaglandin E2 (PGE2) have been considered as important mediator to maintain the podocyte and glomerular function. However, it remains unknown whether COX-2 products from other substrates such as anandamide (AEA) are also important in regulation of podocyte and glomerular function.
- Our recent studies have shown that an AEA product via COX-1, prostamide E2, regulates renal medullary blood flow and sodium excretion exerting an action as a natriuretic factor.

METHODS

Cell culture. Conditionally immortalized mouse podocytes (kindly provided by Dr. Paul Klotman, Division of Nephrology, Department of Medicine, Mount Sinai School of Medicine, New York, New York, USA) were cultured at 33°C on collagen I-coated flasks or plates in RPMI 1640 medium supplemented with recombinant mouse interferon-γ. The cells were then differentiated by culturing at 37°C for 10-14 days in medium without interferon-γ prior to their use in experiments.

Immunofluorescence staining. Cells were grown on coverslips and fixed in PBS containing 4% paraformaldehyde for 10 minutes. Donkey serum (5%) (Sigma, St. Louis, MO) was included in all blocking and primary and secondary antibody buffers. Coverslips were incubated with primary antibodies overnight at 4°C. Secondary antibodies were Alexa Fluor-conjugated (Invitrogen, Inc, Grand Island, NY). Coverslips were mounted in Vectashield reagent containing DAPI (Vector Laboratories, Inc, Burlingame, CA). The fluorescent images were obtained using a confocal laser scanning microscope (Fluoview 1000, Olympus, Japan) and analyzed using Image J software.

Caspase-1 activity, IL-1β and VEGF production. Cell lysate was collected for caspase-1 activity assay (Caspase-1 Colorimetric Assay Kit, BioVision), and cultured medium was collected for IL-1β and VEGF production assay by using commercially available kits (Bender Medsystems, R&D Systems).

RESULTS

Figure 1. Effects of PE2 and AEA on Hcy-induced inflammasome activation. (A) Representative confocal fluorescent images in podocytes after stimulation of Hcy or with PE2 (10 μM, pre-treat 30 minutes) or AEA (10 μM, pre-treat 30 minutes). (B) Summarized co-localization coefficient data showing that co-localization levels of NALP3 with ASC or caspase-1 were attenuated by PE2 but not by AEA. n=6 batches of cells, *P<0.05 vs. Ctrl group, #P<0.05 vs. corresponded Hcy group.

Figure 2. Effects of PE2 and AEA on Hcy-induced augmentation of caspase-1 activity and IL-1β production. (A) Caspase-1 activity measurement and (B) IL-1β production measurement in podocytes with Hcy, Hcy+PE2, Hcy+AEA. It was shown that PE2 but not AEA attenuated Hcy-induced increases in caspase-1 activity and IL-1β production. n=6 batches of cells, *P<0.05 vs. Ctrl group, #P<0.05 vs. Hcy group.

Figure 3. Pretreatment of PE2 preserved podocyte function as observed by fluorescence microscopy of podocin as normal podocyte marker and desmin as podocyte injury marker. PE2 but not AEA blocked Hcy-induced decrease in podocin levels in podocytes (A) and prevented Hcy-stimulated increase in desmin (B). n=6.

Figure 4. Representative microscopic images showing F-actin expression and arrangement in podocytes using rhodamine-phalloidin staining (A). F-actin was found to be aggregated around the periphery of cells after Hcy stimulation. However, the pattern was partially restored by PE2 pretreatment but not by AEA. In addition, Hcy was able to compromise podocyte function, evident by decreased VEGF secretion, which was restored by PE2 but not by AEA (B). n=6, *P<0.05 vs. Ctrl group, #P<0.05 vs. Veh+Hcy group.

Figure 5. Effects of AEA in high concentrations on Hcy-induced NLRP3 inflammasome activation and podocyte injury. High dose of AEA (100 μM) but not low dose was able to significantly inhibit the NLRP3 inflammasome activation as shown by the decrease in caspase-1 activity (A) and IL-1β production (B). In addition, the pretreatment of selective Cox-2 inhibitor, celecoxib (CEL), significantly blocked the effect of high dose of AEA. By VEGF assay, Hcy-induced podocyte dysfunction was also recovered by high dose of AEA, which was inhibited by CEL in COX-2 inhibitor pretreatment. n=6, *P<0.05 vs. Ctrl group, #P<0.05 vs. Veh+Hcy group.

CONCLUSION

Our results provide evidence to support the view that PE2 suppresses Hcy-induced NLRP3 inflammasome activation in podocytes and thereby prevents these cells from Hcy-induced injury and dysfunction.