2019

Norepinephrine Induces Macrophage Polarization Critical to Eliciting Renal Fibrogenesis

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Abstract and Introduction

Renal denervation before ischemic injury has been shown to protect against fibrogenesis and the inflammatory response, which are two causes for the progression of chronic kidney disease. However, the administration of norepinephrine (NE) to denervated renal systems induced fibrogenesis and inflammation after ischemic injury. Our previous data indicates that NE-mediated stimulation of the α2-AR receptors is responsible for regulating several of the processes implicated in fibrogenesis and inflammation, including the accumulation, migration, and infiltration of macrophages to the site of injury; this is especially relevant as macrophages have been implicated as one potential cause for the inflammatory response.

Recent studies, completed in response to the idea that stimulation with clonidine or moxonidine seems to protect against renal fibrogenesis, suggest that NE-mediated stimulation of the α2-AR receptors is responsible for fibrogenesis and inflammation. This is supported by the fact that moxonidine inhibits the release of norepinephrine while simultaneously stimulating the central α2-AR receptors while clonidine is responsible for preventing the release of renin which, as explained by the renin- angiotensin-aldosterone system, prevents the release of norepinephrine from sympathetic nerve endings and the inhibition of its re-uptake. However, there are few studies that explain how the absence of norepinephrine could affect macrophage function.

To define the role that norepinephrine plays in renal fibrogenesis and the inflammatory response after ischemia, three experiments were performed to answer the following questions: 1. Can treating the macrophage cell cultures with LPS induce differentiation between M1 and M2 macrophage phenotypes? 2. Do injured macrophages have the capacity to release norepinephrine? and 3.If so, what is the effect of varying concentrations of norepinephrine on the differentiation between M1 and M2 macrophage phenotypes?

References

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Materials and Methods

Treatment with Lipopolysaccharide (LPS): Macrophage cell cultures (Raw 264.7 cells), were seeded on multiple 12-well plates. After an initial incubation period, the cells were treated for 18 hours with 1 or 10 ng/ml of LPS. To observe the level of NE release, the LPS-treated Raw 264.7 cells in conditioned media were analyzed by ELISA. To quantify the level of M1 and M2 macrophage phenotype expression, mRNA was extracted and quantitative real-time PCR was performed for M1 and M2 phenotype markers (iNOS, IL-6, IL-1β and TNF-α for M1 and IL-10, Arginase-1, and TGF- β for M2).

Treatment with Norepinephrine (NE): Raw 264.7 cells, consisting of macrophage cell cultures, were obtained and seeded on multiple 12-well plates. After an initial incubation period, the cells were treated for 18 hours with 1, 10, or 50 μM of NE. To observe the level of M1 and M2 macrophage phenotype expression, quantitative real-time PCR was performed. Certain M1 and M2 phenotype markers were measured in the mRNA that was extracted (iNOS, IL-6, IL-1β and TNF-α for M1 and IL-10, Arginase-1, and TGF- β for M2). Slides of the Raw 264.7 cells were stained with M1 and M2 phenotypic markers (IL-1β for M1 and TGF-β for M2) to highlight the differences between representative images of the control samples and the Raw 264.7 cells treated with 10 μM of NE.

Preparation of Slides: Raw 264.7 cells were cultured on coverslips and treated for 18 hours with 10 μM of NE. They were subsequently stained with M1 or M2 phenotypic markers (IL-1β for M1 and TGF-β for M2). Representative images of the control samples and the Raw 264.7 cells were then photographed with the microscope.

Treatment of Raw 264.7 Cells with LPS Activates Expression of M1 and M2 Macrophage Phenotypes

The treatment of Raw 264.7 with LPS cells induces M1 macrophage activation.

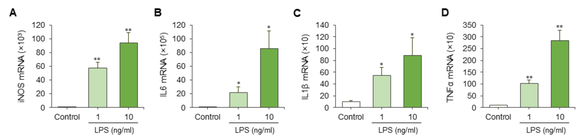

Figure 1. Effect of lipopolysaccharide on the M1 macrophage phenotype. Raw 264.7 cells were treated for 18 h with 1, or 10 ng/ml of lipopolysaccharide. (A-D) The levels of iNOS, IL-6, IL-1β and TNF-α mRNA were determined by quantitative real-time PCR. Expression of all genes is normalized to GAPDH. Results are expressed as the mean ± SE (n=3). *, p<0.5; **, p<0.01. LPS, lipopolysaccharide.

The treatment of Raw 264.7 with LPS cells induces M2 macrophage activation.

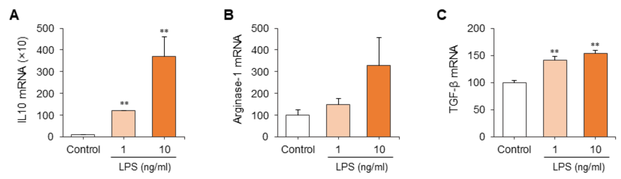

Figure 2. Effect of lipopolysaccharide on the M2 macrophage phenotype. Raw 264.7 cells were treated for 18 h with 1, or 10 ng/ml of lipopolysaccharide. (A-C) The levels of IL-10, Arginase-1, and TGF- β mRNA were determined by quantitative real-time PCR. Expression of all genes is normalized to GAPDH. Results are expressed as the mean ± SE (n=3). *, p<0.5; **, p<0.01. LPS, lipopolysaccharide.

Treatment of Raw 264.7 Cells with LPS Induces Norepinephrine Release

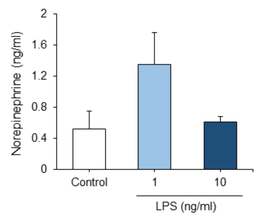

Figure 3. Effect of lipopolysaccharide on norepinephrine release. Raw 264.7 cells were treated for 18 h with 1 or 10 ng/ml of lipopolysaccharide. The level of norepinephrine in LPS-treated Raw 264.7 cells conditioned media was analyzed using ELISA. Results are expressed as the mean ± SE (n=3). *, p<0.5; **, p<0.01. LPS, lipopolysaccharide.

Acknowledgments

I would like to thank Drs. Mi Ra Noh and Hee-Seong Jang for assisting and teaching me the valuable techniques used throughout the course of this research project. I would also like to thank my mentor, Dr. Babu Padanilam, for providing me with his previous research that I used to formulate my research proposal. This work was supported by the National Institutes of Health grants DK-116987 , DK-120533 and the American Heart Association (AHA) Grant in Aid 15GRNT25080031.

Treatment of Raw 264.7 Cells with NE Also Activates Expression of M1 and M2 Macrophage Phenotypes

The treatment of Raw 264.7 with NE induces M1 macrophage activation.

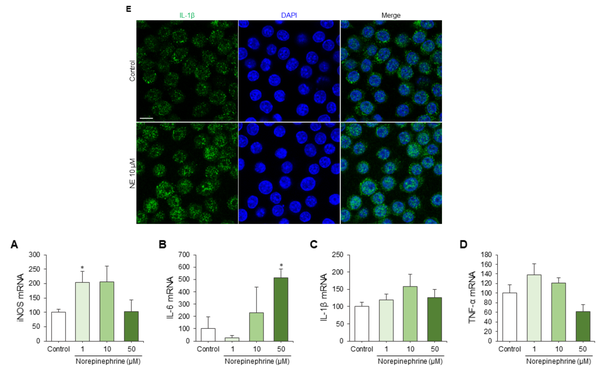

Figure 4. Effect of norepinephrine on the M1 macrophage phenotype. Raw 264.7 cells were treated for 18 h with 1, 10, or 50 μM of norepinephrine. (A-D) The levels of iNOS, IL-6, IL-1β and TNF-α mRNA were determined by quantitative real-time PCR. Expression of all genes is normalized to GAPDH. (E) Representative images of IL-1β in control or 10 μM of norepinephrine treated-Raw 264.7 cell. Results are expressed as the mean ± SE (n=3). *, p<0.5; **, p<0.01. NE, norepinephrine. Scale bar=10 μm.

The treatment of Raw 264.7 with NE induces M2 macrophage activation.

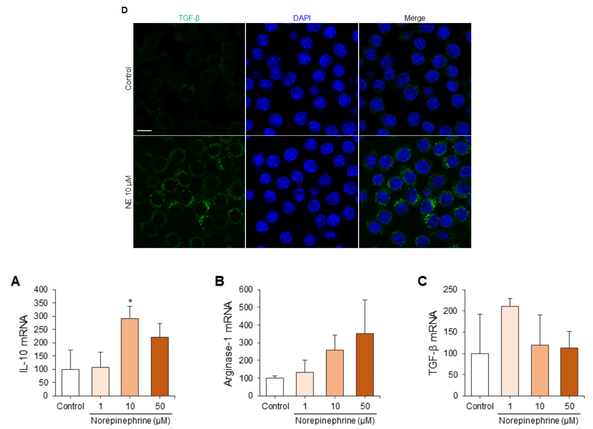

Figure 5. Effect of norepinephrine on the M2 macrophage phenotype. Raw 264.7 cells were treated for 18 h with 1, 10, or 50 μM of norepinephrine. (A-C) The levels of IL-10, Arginase-1, and TGF- β mRNA were determined by quantitative real-time PCR. Expression of all genes is normalized to GAPDH. (D) Representative images of TGF-β in control or 10 μM of norepinephrine treated-Raw 264.7 cell. Results are expressed as the mean ± SE (n=3). *, p<0.5; **, p<0.01. NE, norepinephrine. Scale bar=10 μm.

Conclusions and Future Implications

The results show that LPS, which is used to replicate renal ischemia reperfusion injury, influences both M1 and M2 macrophage phenotype expression in raw cells and induces NE release. The results also show that, like LPS, treatment with NE also influences both M1 and M2 macrophage phenotype expression in raw cells. This suggests that NE may act as an activator for the M1 and M2 macrophage phenotypes after ischemic injury to the renal system, leading to the renal fibrogenesis and inflammation usually seen in chronic kidney disease. Therefore, the inhibition of NE release may represent a novel and effective therapeutic strategy that can be used to prevent renal fibrogenesis and inflammation after acute kidney injury.