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Norepinephrine Induces Macrophage Polarization Critical to Eliciting Renal Fibrogenesis

Hannah Manoj1, Mi Ra Noh2, PhD, Hee-Seong Jang2, PhD, and Babu Padanilam2, PhD: Washington University in St. Louis1; Department of Cellular and Integrative Physiology, University of Nebraska Medical Center, Omaha, NE2

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 may act as an activator for the M1 and M2 macrophage phenotypes? Do injured macrophages have the capacity to release norepinephrine? and 3. If so, the macrophage cell cultures with LPS induce differentiation between M1 and M2 macrophage phenotypes 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?

Materials and Methods

Treatment with LPS (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 10 ng/ml of LPS. To observe the level of NE release, the LPS-treated Raw 264.7 cells in conditioned media were analyzed using 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 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 NE release, the LPS-treated Raw 264.7 cells conditioned media were analyzed using ELISA.

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, 10 ng/ml of lipopolysaccharide. 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.

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 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 (NE). 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. (D) Representative images of TGF-β in control or 10 µM of norepinephrine treated-Raw 264.7 cells. Results are expressed as the mean ± SE (n=3). *, p<0.5; **, p<0.01. NE, norepinephrine. Scale bar=10 μm.

Treatment of Raw 264.7 Cells with LPS Induces Norepinephrine Release

The treatment of Raw 264.7 with LPS cells induces M2 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. Results are expressed as the mean ± SE (n=3). *, p<0.5; **, p<0.01. NE, norepinephrine. Scale bar=10 μm.

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 2. Effect of lipopolysaccharide on the M2 macrophage phenotype. Raw 264.7 cells were treated for 18 h with 1, 10, and 50 µM of lipopolysaccharide. (A-D) 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.

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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.

References

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