

Effects of Exosomes (EXOs) Derived by Renal Pluripotent Stem Cells (rPSCs) on the Cisplatin (Cis) Nephrotoxicity in Mice

Luciana A Reis¹, Adriana A F Carbonel², Carla C Maganhin², Manuel de J Simones² and Nestor Schor¹

¹Nephrology Division, Department of Medicine, UNIFESP/ EPM, São Paulo, Brazil.

²Morphology Department, UNIFESP/ EPM, São Paulo, Brazil

The acute kidney injury (AKI) is characterized by Cis that induces hypoxia and generates free radicals, inflammation and apoptosis of renal tubules [1]. For these reasons it becomes essential to use strategies to prevent this acute damage. Therefore EXOs are potential tools [2]. In this study, we evaluated the EXOs derived by rPSCs on the nephrotoxicity induced by Cis. For in vitro methods, rPSCs mice (C57BL6-J) were used to obtain cultured using standard techniques following glomerular isolation by differential sieving and rPSCs. The rPSCs were characterized by immunofluorescence and FACS utilizing positive antibodies (Wnt1, CD24, PAX2 and ZO1) and negative antibodies (CD45, Thy-1 and pan cytokeratin). MSCs Mice (C57BL6-J) were used to obtained cultured using standard techniques following bone marrow isolation by differential sieving and MSCs. EXOs: rPSCs were incubed for 24 hours. Then, the EXOs were obtained by ultracentrifugation technique and characterized by Western blot utilizing CD9 and CD63. For in vivo methods, mice were treated with Cis (10mg/BW) or PBS (vehicle - CTL group) during 5 days. At the 3th day of this treatment, animals were treated with EXOs (35µg/ml) derived from rPSCs in only one injection. At the end of these treatment, urine 24 hours and blood were collected by biochemical and cytokines (IL2, IL6 and IL10) analysis and kidney to evaluated for HE, caspase 3 and KI67. Results: It was observed that the rPSCs positive for the markers scored (Wnt1, CD24, PAX2 and ZO1); EXOs marked to CD9 and 63. The creatinine, urea, IL2 and IL6 increased in Cis group when compared to CTL; Cr:(2.5±0.9 vs. 1.4±0.4 mg/dl; p<0.05); U: (300±20.6 vs. 100±22.8 mg/dl; p<0.05); IL2: (1.2±0.3 vs. 0.2±0.02 pg/ml; p<0.05); IL6: (1.8±0.4 vs. 1.1±0.07pg/ml; p<0.05); Therefore, t's not observed difference in IL10 (0.2±0.01 vs. 0.2±0.03pg/ml; p<0.05). When these animals received EXOs it was observed a decrease in Cr, U, IL2, IL6 and a significant increase of anti-inflammatory cytokines IL10 when compared to Cis group; Cr:(1.4±0.7 vs. 1.4±0.4 mg/dl; p<0.05); U: (208±32.7 vs. 100±22.8 mg/dl; p<0.05); IL2: (0.4±0.01 vs. 0.2±0.02 pg/ml; p<0.05); IL6: (0.7±0.5 vs. 1.1±0.07pg/ml; p<0.05); Therefore, t's not observed difference in IL10 (1.1±0.02 vs. 0.2±0.03pg/ml; p<0.05). In Cis-group, the kidneys showed a small marked KI67 and intensive caspase 3 and acute tubular necrosis (ATN) expression but differently, it was highly marked for KI67 and lower expression for caspase 3 in Cis+EXOs groups and no histological ATN lesions were observed. These results strongly suggest that EXOs derived from rPSCs can minimize AKI induced by Cis. These therapeutics EXOs effects have a significant impact on renal function and holds substantial potential use especially by avoiding transplanting cells with potential adverse effects, in this experimental model.

References:

[1] Dos Santos OF, Boim MA, Barros EJ, Schor N. *Kidney Int.* 1991; 40(4):742-7.

[2] Reis LA, Borges FT, Simões MJ, Borges AA, Sinigaglia-Coimbra R, Schor N. *PLoS One.* 2012; 9:e44092

Figure 1: HE [μm^2] in mice (C57BL-6J) with AKI induced by Cis

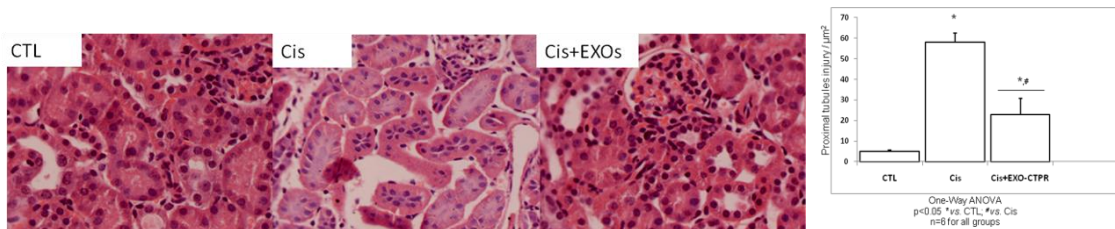


Figure 2: KI67 [μm^2] in mice (C57BL-6J) with AKI induced by Cis

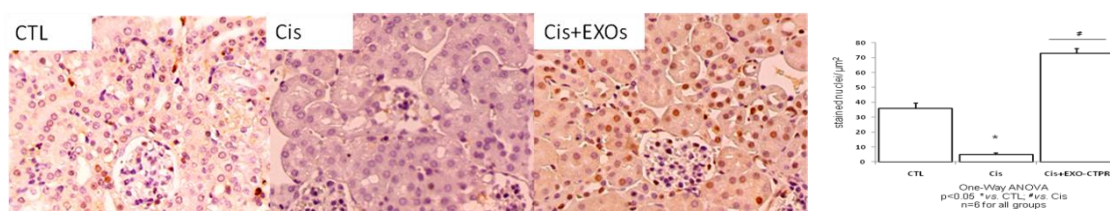


Figure 3: Caspase 3 [μm^2] in mice (C57BL-6J) with AKI induced by Cis

