CONDITIONED MEDIUM (CM) FROM MESENCHYMAL STEM CELLS (MSCS) PROTECTS HUMAN PROXIMAL TUBULAR CELLS (HK2) LESIONS BY LPS.

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INTRODUCTION

Recent studies emphasize the contribution of MSCs in the regeneration related to acute kidney injury (AKI). MSCs mitigate the damage and / or accelerate the repair and participate in the immunomodulation processes, probably by paracrine pathways. This protection involves the CM that contains soluble factors or microvesicles (MVs). MVs are microparticles involved in intercellular communication mechanism. Its function is related to load carrying as microRNAs and mRNA.

OBJECTIVE

We studied the paracrine effect of MSCs in vitro models of AKI in sepsis, caused by application of *Escherichia coli* lipopolysaccharide (LPS) in cultured human immortalized proximal tubular cells (HK2).

MATERIALS AND METHODS

Human proximal tubular cells (HK2) were cultured and treated for 24-96 h with LPS (100mg/ml), with or without conditioned medium (CM) from MSCs. The CM was also treated (est) with LPS (100mg/ml) and gentamicin (GENTA - 2 mM) for 48 h before the experiment and it was employed in frozen form (CMc). Cell proliferation was evaluated by MTT assay.

CONTROL HK2 cells	LPS HK2 cells+ LPS	CM HK2 cells + CM	LPS + CM HK2 cells+ LPS + CM	CMc HK2 cells + CMc

LPS + CMc HK2 cells+ LPS + CMc	CM est LPS HK2 cells+ CM est LPS	CM est LPS + LPS HK2 cells + CM est LPS + LPS	CM est GENTA HK2 cells + CM est GENTA	CM est GENTA + LPS HK2 cells + CM est GENTA + LPS

RESULTS AND CONCLUSION

We observed a significant decrease in apoptosis (13.1 ± 2.5% vs. 30.2 ± 4.5%, p <0.05) and necrosis (17.4 ± 4.7% vs. 52.6 ± 5 3%, p <0.05) between the MC + LPS group compared with the group treated only with LPS. And we observed an increase in cell proliferation in the CM + LPS group compared with the group treated only with LPS (0.12 ± 0.02 DO vs 0.10 ± 0.02 DO, p < 0.05), and the major proliferation happened with the CMc + LPS (0.16 ± 0.04 DO, p < 0.05). When comparing the groups that received CM stimulated with drugs it was observed higher proliferation in CM (with GENTA) + LPS (0.16 ± 0.05 DO, p < 0.05) than in MC (with LPS) + LPS (0.09 ± 0.02 DO, p < 0.05), the latter result was very similar to the LPS group. Comparing only the different CM with HK2 cells in culture, it was measured greater proliferation in CM (with LPS) (0.27 ± 0.07 DO, p < 0.05) when compared with CM and CMc (0.21 ± 0.03 DO vs. 0.20 ± 0.06 DO, p < 0.05). CM (with GENTA) group showed the lowest proliferation compared with others (0.17 ± 0.02 DO, p < 0.05). These preliminary results suggested that CM from MSCs exerts a protective effect by stimulating cell proliferation in cells during the treatment with LPS. Also,it is also possible to "conditioned" the CM and stimulate enhancing it potential effects. Moreover, results may indicate that it is possible to minimize AKI induced by LPS without introducing stem cells but only with their CMs, avoiding potential harmful effects of cell therapy. It is clear that more studies are needed to enable the use of CMs stimulated and frozen.

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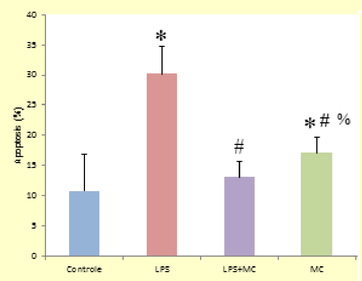
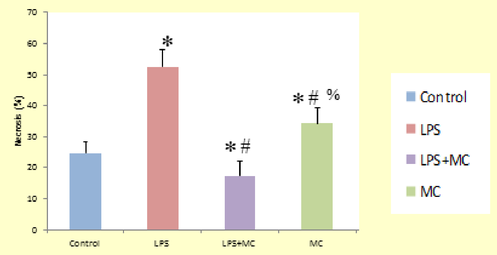
Study of the Effect of the conditioned medium of MSCs in HK2 cells treated with LPS - Hoe



Control; LPS = HK2 cells treated with LPS for 24 hours; LPS + MC = HK2 cells treated with LPS for 24 hours and medium of MSCs; MC = HK2 cells treated with MSC medium for 24 hours.
* Vs Control; # Vs LPS; % Vs LPS+MC; & Vs MC

Study of the Effect of the conditioned medium of MSCs in HK2 cells treated with LPS - AO



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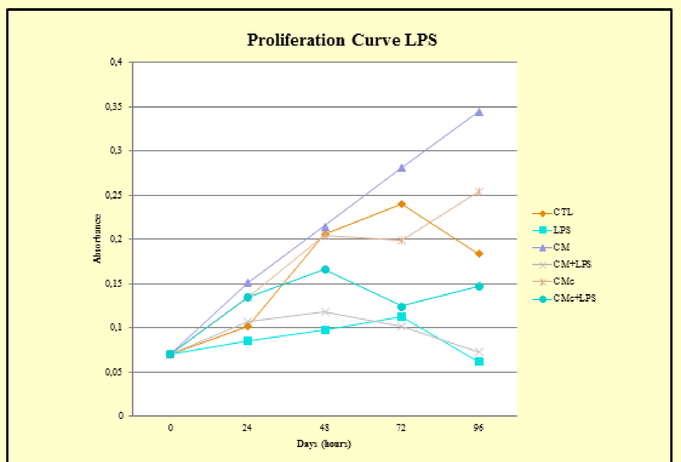
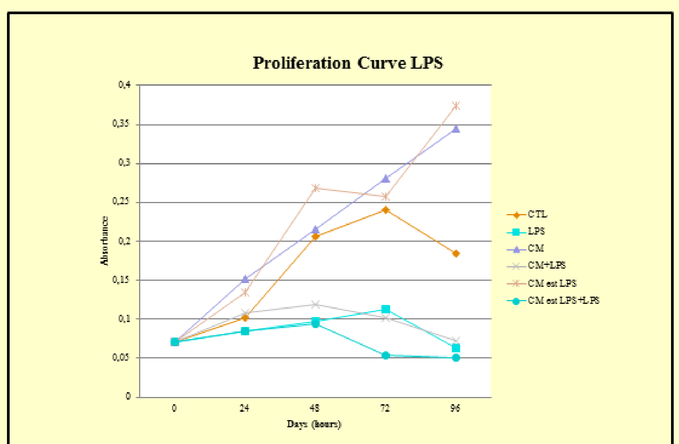


CTL = Control; LPS = HK2 cells treated with LPS; CM = HK2 cells treated with medium from MSCs; CM + LPS = HK2 cells treated with LPS and medium from MSCs; CMc = HK2 cells treated with medium frozen from MSCs; CMc + LPS = HK2 cells treated with LPS and medium frozen from MSCs.



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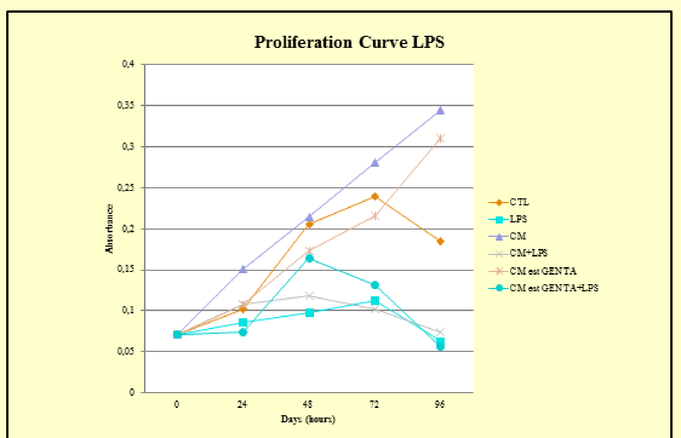

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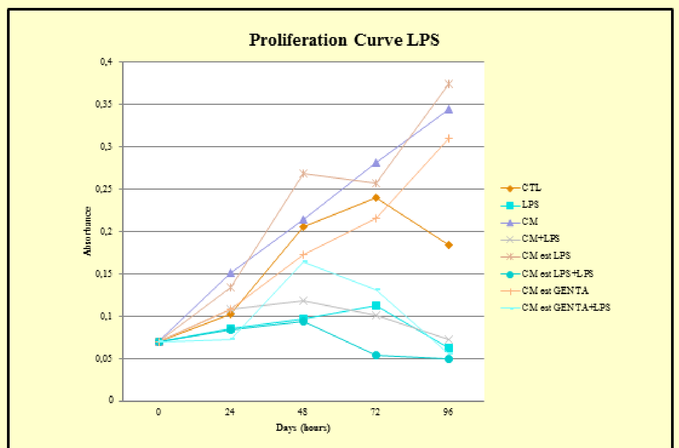

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