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Review Article

The Conditioned Medium of Umbilical Cord Mesenchymal Stem Cells Diminished the Traumatic Effects of HUVECs Injured by Indoxyl Sulfate

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Abstract

Cardiovascular Disease (CVD) is the leading cause of mortality in Chronic Kidney Disease (CKD) patients. Extracellular Vesicles (EVs) secreted by Mesenchymal Stem Cells (MSCs) are known to exert therapeutic effects on cell/tissue regeneration. In this study MSCs were bioprocessed by serum deprivation, which produced a bioactive secretome in Conditioned Medium (CM). MSC-CM was applied to treat human umbilical vein endothelium cells (HUVECs) injured by the uremic toxin indoxyl sulfate (IS). Based on our results, administration of MSC-CM with 48 h of serum deprivation demonstrated great potential for the treatment of injured HUVECs. MSC-CM may be developed into a class of biopharmaceuticals for the treatment of CVD in CKD.

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Introduction

Mesenchymal stem cells (MSCs) are the most common cell therapy products used in regenerative medicine. Nevertheless, there are safety concerns in MSC therapy regarding immune reactions, cancer development, tumor metastasis, tissue calcification, etc [1-3]. It has been proven, however, that MSCs exert therapeutic effects by the secretion of beneficial factors and vesicles for tissue regeneration [4]. Furthermore, Extracellular Vesicles (EVs) secreted by MSCs exhibit the biological properties of their parent cells [5] and have been successfully applied for treating a broad range of diseases [6,7] without the many drawbacks of cell therapy. Cardiovascular Disease (CVD) is an important cause of increasing mortality in chronic kidney disease (CKD) patients with a prevalence rate as high as 63% The protein-bound uremic toxin, Indoxyl Sulfate (IS), which is normally excreted in the urine, is one of several known nephrovascular toxins that contribute to high cardiovascular risk and mortality in CKD [8-10]. Since effective medicines are lacking for the treatment of CKD-related CVD, we conducted a study to survey the therapeutic efficacy of MSC-Conditioned Medium (CM), which is rich in secreted EVs. CM from Umbilical Cord (UC)- derived MSCs was prepared by different manufacturing processes. Then the CM was used to treat IS-injured Human Umbilical Vein Endothelium Cells (HUVECs). MSC-CM prepared by different processes exerted dissimilar biological activity, MSC-CM with 48 h of serum deprivation produced the most significant protective effect. Our results suggest that CM from UC-MSCs has the potential to become a biopharmaceutical reagent in the future.

Materials and Methods

Umbilical cord-derived MSC (UC-MSC) culture and treatments

Normal human UC-MSCs were purchased from ATCC (PCS-500-010, ATCC, Manassas, VA). Cells were grown in MSC Basal Media supplemented (PCS-500-030, ATCC, Manassas, VA) with the MSC Growth Kit (PCS-500-040, ATCC, Manassas, VA) and were incubated at 37 °C, 5% CO2. The serum supplement to the culture medium was pre-processed by the Exosome Depletion Kit (Cat# 61200, Norgen Biotek Corp., Canada) to ensure all EVs in the medium were from UC-MSCs. UC-MSCs were propagated to passage 10 and then divided into two batches, one with and one without serum deprivation. MSC-CM was collected at 24 and 48 h thereafter. The collected

MSC-CM was centrifuged for 5 min and then passed through a 0.22 μm filter before use.

HUVEC culture and treatments

HUVECs were purchased as part of the Clonetics™ Endothelial Cell System (Lonza Walkersville, Inc., MD, USA; Cat.no. C2519A). Cells were cultured in EGMTM-2 BulletKitTM medium (Cat.no. CC-3162, Lonza), which contained EBMTM-2 Basal Medium (Cat. no. CC-3156, Lonza) and EGMTM-2 SingleQuotsTM Supplements (Cat.no. CC-4176, Lonza) incubated at 37 °C, 5% CO2. Fetal bovine serum (FBS) was processed by the Exosome Depletion Kit (Cat# 61200, Norgen Biotek Corp., Canada) before addition to the cell cultures. HUVECs from the fifth passage were incubated in 0.1 mM IS (Cat.no. I3875, Sigma-Aldrich, Germany) concomitant with four different samples of MSC-CM (with/without serum deprivation for 24 or 48h) as various proportions of the culture medium, as shown in Table 1.

UC-derived MSC-CM				
Serum Deprivation	(+)	(-)	(+)	(-)
Time of fasting (h)	24	24	48	48
Proportion	10%	10%	10%	10%
HUVEC Culture	20%	20%	20%	20%
Medium Volume	30%	30%	30%	30%

Table 1: Bioprocessing of UC-derived MSC-CM and proportion of the HUVEC culture medium volume.

Cell proliferation assay

HUVECs were first cultured in a 6-cm dish; then the fifth passage cells were re-seeded in a 96-well microplate at a density of 2,500 cells/well and were incubated in exosome-free culture medium containing 0.1 mM IS for 96 h. HUVEC proliferation was measured by 3-(4,5-Dimethylthiazol-2-yl)-2,5- diphenyl tetrazolium bromide (MTT) (EMD Biosciences, USA) assays at timepoints of 0 and 96 h (n=3) according to the manufacturer's instructions. The absorbance was detected at 570 nm.

Results

Four samples MSC-CM produced by different bioprocessing methods exhibited different protective efficacies against IS injury in HUVECs. MSC-CM produced after 24 h either with or without serum deprivation had no influence on HUVEC proliferation (Figure 1A&1B). However, MSC-CM produced after 48 h with or without serum deprivation exerted a significant protective effect on the proliferation of HUVECs injured by IS. MSC-CM produced under serum deprivation for 48 h, compared to the serum-supplemented group, provided a stronger impact on HUVECs with IS injury, particularly when applied as 10% of the culture volume (Figure 1C&1D).

Discussion

In this study, we analyzed how different manufacturing procedures, such as duration of cell conditioning or selection of serum-free culture medium, might influence the composition of human MSC-CM as well as its biological activity. The results revealed that 48 h under serum-free culture conditions produced MSC-CM with a significant therapeutic benefit for the treatment of IS-injured HUVECs. The level of benefit was dose-dependent, as evident from the different results at different proportional volumes. Exosomes are the smallest class

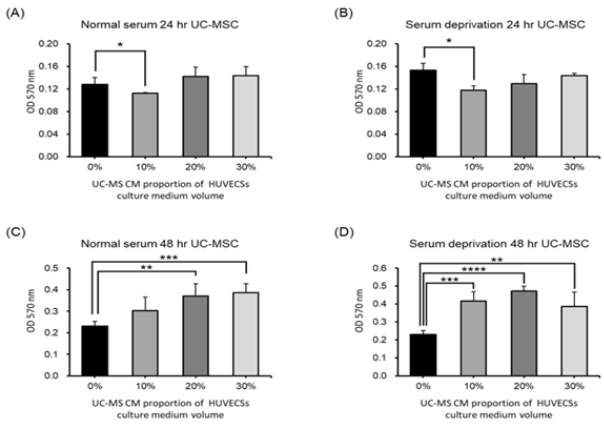

Figure 1: Effect of MSC-CM on IS-injured HUVECs. MSC-CM was collected via various bioprocessing methods (24 or 48 h with or without serum deprivation) and added in different proportions (10%, 20%, 30%) to the HUVEC culture medium with 0.1 mM IS for 4 days. Proliferation of HUVECs was examined by MTT assays. The cell growth ratio was expressed as relative absorbance compared with 0 h time point. (A) MSC-CM without serum deprivation, 24 h. (B) MSC-CM with serum deprivation, 24 h. (C) MSC-CM without serum deprivation, 48 h. (D) MSC-CM with serum deprivation 48 h. * p <0.05; ** p <0.01; *** p <0.001; ****<0.0001.

of EVs, but still contain bioactive components, such as cell signaling proteins and RNAs, which influence cell communication [11-14]. FBS/serum used to supplement culture media is rich in exosomes, which can affect in vitro EV analyses [15]. To prevent confounding, FBS/serum should be pre-processed with exosome depletion kits to produce EV-depleted FBS/serum. CVD is the leading cause of mortality in CKD patients when non- traditional risk factors are surveyed, one that appears is uremic toxins such as IS [16]. MSC-CM provides an opportunity in the treatment of CVD, such as alleviation of endothelial dysfunction to preserve brain tissue and reduction of irradiation-induced damage in cardiac fibroblast cells [17-18]. However, there has been no research applying CM to IS-induced CVD. With the potential of MSC-CM's reparative ability, as our study results revealed, it may constitute an alternative treatment modality in the future, after definition of the precise molecules contained in the MSC-CM.

Conclusion

To date, MSC-CM has been applied to divergent diseases and many studies have shown its therapeutic effects [6]. Our study demonstrated that different processing methods can produce MSC-CM with different protective effects. To maximize the impact of the UC-MSC secretome, further evaluation of MSC-CM content under different bioprocessing methods will be required prior to its application as a treatment for various diseases.

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Author's Contribution

YCH and LCL conceived and designed the experiments. YCH, CHC, PHK, KTC performed the experiments. YCH, PHK, TCT, LCL analyzed the data. YCH, PKH, CWH and LCL contributed reagents, materials or analysis tools. YCH and LCL wrote the paper. All authors reviewed the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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