EFFECTS OF BACTERIAL LIPOPOLYSACCHARIDE AND SHIGA TOXIN ON INDUCED PLURIPOTENT STEM CELL-_DERIVED MESENCHYMAL STEM CELLS

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ABSTRACT—Background: Mesenchymal stem cells (MSCs) can be activated by different bacterial toxins. Lipopolysaccharides and Shiga Toxin (Stx) are the main toxins necessary for hemolytic uremic syndrome development. The main etiological event in this disease is endothelial damage that causes glomerular destruction. Considering the repairing properties of MSC, we aimed to study the response of MSC derived from induced pluripotent stem cells (iPSC-MSC) to LPS and/or Stx and its effect on the restoration of injured endothelial cells. Methods: iPSC-MSC were treated with LPS and/or Stx for 24 h and secretion of cytokines, adhesion, and migration were measured in response to these toxins. In addition, conditioned media from treated iPSC-MSC were collected and used for proteomics analysis and evaluation of endothelial cell healing and tubulogenesis using human microvascular endothelial cells 1 as a source of endothelial cells. Results: The results obtained showed that LPS induced a proinflammatory profile on iPSC-MSC, whereas Stx effects were less evident, even though cells expressed the Gb3 receptor. Moreover, LPS induced on iPSC-MSC an increment in migration and adhesion to a gelatin substrate. Addition of conditioned media of iPSC-MSC treated with LPS + Stx decreased the capacity of human microvascular endothelial cells 1 to close a wound, and did not favor tubulogenesis. Proteomic analysis of iPSC-MSC treated with LPS and/or Stx revealed specific protein secretion patterns that support the functional results described. Conclusions: iPSC-MSC activated by LPS acquired a proinflammatory profile that induces migration and adhesion to extracellular matrix proteins but the addition of Stx did not activate any repair program to ameliorate endothelial damage, indicating that the use of iPSC-MSC to regenerate endothelial injury caused by LPS and/or Stx in hemolytic uremic syndrome could not be the best option to consider to regenerate a tissue injury.

KEYWORDS—Mesenchymal stem cells; endothelial injury; bacterial toxins; tissue regeneration; hemolytic uremic syndrome

INTRODUCTION

Mesenchymal stem cells (MSC) are multipotent cells associated with the treatment of different pathologies due to their regenerative properties, thus providing an interesting therapeutic option for various diseases, mainly those that are present in an inflammatory response and tissue damage, considering its use for clinical applications (1).

Isolating MSCs and obtaining a considerable number for handling often present difficulties. In this sense, derivation of MSCs from induced pluripotent stem cells (iPSCs) is a widely accepted method that results in cells that have similar properties to those obtained directly from adult tissues. Protocols for obtaining MSCs from iPSC are increasingly being validated within the scientific community as an alternative to obtain MSCs. In this sense, our group described a robust and fast method to obtain the iPSC-MSCs that were used in this work (2). Luzzani and Miriuka’s group showed that iPSC-derived MSCs closely resemble adult tissue-derived MSCs, particularly those obtained from umbilical cord (Wharton jelly), showing ability to differentiate to multiple lineages and to have immunomodulatory effects (2,3). Altogether, these evidences indicate that iPSC-derived MSCs constitute a reliable and consistent source of MSCs.

Moreover, many authors described that MSCs are involved in immune processes and participate in the repair of many types of tissue injuries, mainly in a paracrine fashion by secreting numerous soluble factors (4). In addition, it is widely reported the capacity of MSC to migrate into injured sites and to release inflammatory and growth factors (5,6), and in general, these processes could respond to the presence of bacterial toxins. In this sense, it has been described that MSCs can be polarized in vitro toward either pro- or anti-inflammatory phenotypes, depending on the toll-like receptor ligand involved in their activation (7,8). However, if the
inflammatory response turns out to be excessive, tissue damage repair may not be possible (1).

Hemolytic uremic syndrome (HUS) is a disease caused by infections with enterohemorrhagic gram-negative bacteria that produce Shiga toxin (Stx). This toxin interacts with a globotriaosylceramide glycolipid receptor (Gb3) in target cells that usually culminates with the inhibition of protein synthesis and cell death. Endothelial cell damage is a central event in the pathophysiology of HUS and is the most important factor of the microangiopathic process typically found in this disease. The precise and detailed mechanisms of the pathophysiology of HUS have not been precisely elucidated. Different authors agree that the effect of Stx is focused on the endothelial damage of the renal glomerulus probably due to the high concentration of Gb3 in these cells (9). However, there is important evidence that different cytokines induced by Stx itself or by LPS accompany this type of infection and are necessary for the full development of the effect of Stx, often potentiating the endothelial dysfunction (10–12). Taking into account that in many infections, iPSC-MSC can be activated because of bacterial toxins and this can be decisive for recovering homeostasis and repair tissue damage because of their regenerative properties, the aim of this work was to investigate whether LPS and/or Stx treatments modify some iPSC-MSC functions, modulating the characteristic endothelial injury, induced in the context of HUS.

MATERIALS AND METHODS

Cell cultures and treatments

The iPSC-MSC were obtained and differentiated as previously published (2). These cells were maintained using alpha-MEM medium (Gibco, Dublin, Ireland) supplemented with platelet lysate, 10% of penicillin-streptomycin and glutamine (Gibco, Dublin, Ireland). At 80% of confluence, cells were trypsinized with 0.25% of trypsin-EDTA (Gibco, Dublin, Ireland).

Human dermal microvascular endothelial cells 1 (HMEC-1) were used to perform the experiments of endothelial damage. Cells were cultured at 37°C in a 5% CO2 humidified atmosphere using MCDB-131 medium (Gibco, Dublin, Ireland). At 80% of confluence, cells were trypsinized with 0.25% of trypsin-EDTA (Gibco, Dublin, Ireland).

These cells were plated at subconfluency, treated for 24 h, and then gently washed to remove dead cells. After that, the remaining attached cells were fixed and dyed for several washes with 0.1% crystal violet in 20% methanol. Several washes were done to eliminate the residual dye. Then, the crystals were solubilized with 30% acetic acid and measured with an ELISA detector at 540 nm.

Proliferation

A total of 1 × 10^5 iPSC-MSC cells were seeded in 96 well plates with LPS and/or Stx for 48 h at 37°C in 5% CO2. Then, 0.5 μU/well of 3H-thymidine was added and incubated for another 20 h. After that, cells were harvested, scintillation fluid was added, and the radioactive thymidine incorporated into DNA was measured.

Migration assay

The iPSC-MSC (0.25 × 10^5 cells/well) were seeded to confluence in 48-well plates (Jet Biofil) and were incubated with the CM from treated iPSC-MSC immediately after doing the scratch. Starting point (time 0) of the experiment was defined as the moment of CM addition, with an end point of 18 h. Images were captured at both instances with a Nikon Eclipse TS 100 microscope and then analyzed with ImageJ software. We used the freehand tool to manually draw over the gap edges to determine the area of the wound at time 0 and 18 h. The percentage of gap closure was calculated as: [(gap area at 0 h – gap area at x h)/gap area at 0 h] × 100.

Wound repair assay

HMEC-1 (0.25 × 10^5 cells/well) were seeded to confluence in 48-well plates (Jet Biofil) and were incubated with the CM from treated iPSC-MSC immediately after scratching. Images were captured at both instances with a Nikon Eclipse TS 100 microscope and then analyzed with ImageJ software. We used the freehand tool to manually draw over the gap edges to determine the area of the wound at time 0 and 18 h. The percentage of gap closure was calculated as: [(gap area at 0 h – gap area at x h)/gap area at 0 h] × 100.

Adhesion assay

Adhesion of iPSC-MSC was evaluated on 96-well plates previously coated with 2% gelatin (40 min at room temperature, Sigma, USA). Cells were first treated with vehicle (control), LPS, Stx, and LPS + Stx and then collected with trypsin, counted and seeded in the gelatin-coated wells (20,000 cells/well). Cells were allowed to attach for 15 min at 37°C and nonadhered cells were eliminated by vigorous washing. Remnant gelatin-adhered cells were stained with crystal violet solution for 20 min, washed and allowed to dry. Images of adhered cells were captured using a Nikon Eclipse TS 100 microscope and quantified with the ImageJ software using the count cell option.

Gb3 measurement by thin layer chromatography

Gb3 levels were detected by thin-layer chromatography and analyzed by densitometry. The iPSC-MSC cells were cultured in flasks and grown at 37°C in an atmosphere of 5% CO2 until cells were nearly confluent. Cells were treated with Stx and/or LPS as previously described. From each treatment, total cell glycolipids were extracted according to the method of Bligh and Dyer et al. (14). Briefly, 3 mL of chloroform:methanol 2.1 vol/vol were incorporated into the cells, and during 15 min, cells were incubated on ice. Two milliliters of chloroform:water (1:1) were added and centrifuged at 3,000 rpm for 5 min to separate phases. The lower phase, corresponding to the neutral glycolipid extract, was brought to dryness and used for analysis. Gb3 measurement was performed using a purified glycosphingolipid standard.

Conditioned media

The iPSC-MSC were seeded in alpha-MEM and treated with LPS and/or Stx during 24 h. Then, conditioned media (CM) were collected and incubated for 2 h with an anti-Stx antibody (anti-Stx2 variant from Toxin Technology, USA) to block the direct effect of Stx or Polymyxin (Sigma, USA) to block the direct effect of LPS. In LPS + Stx CM, both anti-Stx (10 μg/mL) and Polymyxin (7 μg/mL) were used.

Viability assays

Cells were plated at subconfluency, treated for 24 h, and then gently washed to remove dead cells. After that, the remaining attached cells were fixed and dyed for 20 min using a solution of 0.1% crystal violet in 20% methanol. Several washes were done to eliminate the residual dye. Then, the crystals were solubilized with 30% acetic acid and measured with an ELISA detector at 540 nm.

ELISA assays

Detection of TNF-α (Biolegend, cat. 430,205), IL-8 (Biolegend, cat. 78,141), TGF-β (Biolegend, cat. 436,707), and IL-10 (Biolegend, cat. 430,601) in the supernatants from treated iPSC-MSC was performed using ELISA kits, following manufacturer’s recommendations. Concentration results were obtained in picograms per milliliter.

Tubulogenesis assay

Assays were performed on 96-well plates coated with gelatex at 37°C for no less than 30 min. Approximately 15,000 HMEC-1 cells/100 μL were seeded on coated wells using EGM-2 media (Lonza, Switzerland) and incubated
overnight either with CM from toxin-treated iPSC-MSC or vehicle at 37°C. Images of tubule formation were captured at 24 h using a Nikon Eclipse TS100 microscope followed by analysis on ImageJ software using the count the branch points option.

**Mass spectrometry (MS)**

Liquid chromatography with tandem mass spectrometry assays and mass spectrometry (MS) analysis on CM of three technical replicates from vehicle (control), LPS, LPS + Stx, and Stx-treated iPSC-MSC were performed at the Proteomics Core Facility CEQUIBEM (University of Buenos Aires, Buenos Aires, Argentina) following specifications detailed in La Greca et al. (2018) (5). Briefly, peptides were reduced with dithiothreitol, precipitated with trichloroacetic acid, and digested with trypsin. Approximately, 1 μg of protein digests were analyzed by nano Liquid chromatography with tandem mass spectrometry in a Thermo Scientific QExactive Mass Spectrometer coupled to a nano-HPLC EASY-nLC 1000. Data acquisition and configuration for peptide identification were achieved with XCalibur 3.0.63 software ([www.thermofisher.com/order/catalog/product/OPTON-30965](http://www.thermofisher.com/order/catalog/product/OPTON-30965)) and raw data produced were fed into Proteome Discoverer software to classify identified peptides against Homo sapiens protein sequences database (trypsin specificity) and quantify abundance (area under the curve strategy).

**Bioinformatic analysis of MS data**

Protein abundance obtained from Proteome Discoverer in the form of area-based quantification (area under the curve) (15) was used for downstream analysis. Technical replicates were collapsed and samples normalized by total area (total area per sample/1000). Peptide abundance identified as ALBUMIN (P02768) was excluded from further analysis as it is most likely a residual contaminant from the platelet lysate used during iPSC-MSC routine culture. The rest of the identified proteins were clustered using a hierarchical-based approach and plotted in a heatmap using pheatmap package in R ([r-proyect.org](http://r-proyect.org)). To aid visualization of identified proteins, ProteinIDs were mapped to Gene Names using the uniprotID converter ([http://www.uniprotorg.org](http://www.uniprot.org)). Ontological terms classified as “Biological processes” (BPs) were determined using DOSE (16) and clusterProfiler (17) packages (bioconductor.org) keeping only the top 10 statistically significant overrepresented terms (P < 0.01, q < 0.05). These overrepresented BPs—also called enriched—were determined by statistically testing (Fisher exact test followed by hypergeometric distribution test to evaluate significance) the relationship between the frequency of genes/proteins annotated to the same term (expected or background frequency). Ultimately, this means that enriched BPs showed observed frequency values higher than their expected frequency for that term, and the difference proved to be significant (P < 0.01).

**RESULTS**

**Gb3-expressing iPSC-MSCs remained viable after LPS and Stx treatments**

To establish the concentrations of Stx and LPS to be used with iPSC-MSC and endothelial cells, we set two-dose response curves with different concentrations. As a first step in determining the Stx concentration needed to cause endothelial damage, we treated human microvascular endothelial cells (HMEC-1) with different doses of this toxin and measured the resultant viability after 24 h. As shown in Figure 1A, we found that concentrations of 5, 10, and 20 ng/mL of Stx alone were sufficient to cause endothelial cell death in a dose dependent manner. Lipopolysaccharides did not show any additional toxic effect when combined with Stx. Although various different concentrations of LPS alone did not induce HMEC-1 cytotoxicity (Supplemental Digital Content 1A, http://links.lww.com/SHK/B675), 500 ng/mL of LPS modulated endothelial cell functions by increasing ICAM-1 expression (Supplemental Digital Content 1B, http://links.lww.com/SHK/B675). Both concentrations of LPS and Stx were chosen based on evidence previously published (18). In contrast to the results observed in endothelial cells, none of the concentrations of Stx or Stx in combination with LPS (LPS + Stx) affected iPSC-MSC viability (Fig. 1B), or their proliferation measured by 3H-thymidine incorporation (Fig. 1C). Moreover, the addition of Px to control group did not show significant statistical differences in the viability of iPSC-MSC compared with control group alone (data not shown).

In addition, because Stx did not affect iPSC-MSC viability or proliferation levels, we decided to determine the presence of the
Gb3 receptor in these cells. Using thin-layer chromatography, we observed that iPSC-MSC have the Gb3 receptor, and its level of expression was similar in both Control and treated conditions (LPS, Stx, and LPS + Stx) (Fig. 1D).

These results indicate that even though iPSC-MSC expresses the Stx receptor, the treatment with this toxin alone or in combination with LPS does not affect cell viability, in contrast to what was observed for endothelial cells.

**Lipopolysaccharides induced a proinflammatory program on iPSC-MSC but not Stx**

The iPSC-MSC regulate their microenvironment releasing different cytokines that can modulate BPs in an autocrine or paracrine way (19). Moreover, inflammatory signals released in many infections are associated with migration, adhesion to the extracellular matrix (ECM), and repair mechanisms (8). Therefore, to determine the immunomodulatory contribution of LPS- or Stx-treated iPSC-MSC, we measured the release of the proinflammatory cytokines IL-8, and TNF-α, and the anti-inflammatory cytokines TGF-β and IL-10. As shown in Figure 2, A and B, only LPS significantly increased the release of IL-8 and TNF-α compared with control cells. The addition of Stx alone did not induce the release of IL-8 or TNF-α. When iPSC-MSC were exposed to LPS + Stx, they increased the production of IL-8 and TNF-α compared with control cells, but TNF-α levels were lower compared with LPS alone. In addition, the presence of LPS, Stx, or LPS + Stx decreased significantly the levels of TGF-β compared with control cells (Fig. 2C). Both IL-10 and VEGF levels remained undetectable in all conditions (<15.6 and <31.3 pg/mL, which are the lower detectable concentrations with ELISA kits, respectively). The addition of Px to control group did not show significant statistical differences in the secretory profile from iPSC-MSCs compared with control group alone (data not shown). The results obtained indicate that LPS and Stx polarizes iPSC-MSC toward a proinflammatory phenotype, by increasing pro-inflammatory or decreasing anti-inflammatory cytokines released by iPSC-MSC.

**Lipopolysaccharides and not Stx increased the migration of iPSC-MSC**

It is known that in some inflammatory pathologies, iPSC-MSC can respond to a wide range of extracellular signals and modulate some of their functions (7,8). In this sense, we investigated the effect of LPS and Stx on the capacity of iPSC-MSC to migrate after a wound was performed. We observed that LPS treatment increased the percentage of migration of iPSC-MSC compared with control cells (Fig. 3). Conversely, Stx did not modify this function showing similar migration profiles as in basal condition. The addition of Px to control group did not show significant statistical differences in the migration of iPSC-MSCs compared with control group alone (data not shown).

The combination of LPS + Stx increases these percentages similar to LPS alone when compared with control and Stx treated cells. In conclusion, the inflammatory stimulus LPS increases the migration of iPSC-MSC, whereas Stx does not modify this effect.

**The combination of LPS + Stx augmented iPSC-MSC adherence to the ECM**

The fact that iPSC-MSC migrate sensing inflammatory signals involves a first step of adhesion to the ECM to reach the site of damage (20). In this sense, iPSC-MSC were used for measuring adhesion to a substrate (gelatin) 24 h after incubation with LPS, Stx, or LPS + Stx. Treated cells were harvested and settled on gelatin covered wells. We found that LPS or Stx alone showed minor effects, but the combination of both toxins (LPS + Stx) caused a statistically significant increase in cell adhesion (Fig. 4). The addition of Px to control group increased the adherence to the gelatin matrix compared with control group alone, but it does not affect the main result that was observed in the iPSC-MSC treated...
with LPS + Stx because this group was not added Px. The set of these data suggests that both LPS and Stx together are necessary to increase adhesion of iPSC-MSC.

**Conditioned media from iPSC-MSC exposed to LPS + Stx decreased the capacity to repair endothelial damage**

To investigate the effect of iPSC-MSC previously exposed to LPS and/or Stx on endothelial repair, we performed a wound healing assay using CM from treated iPSC-MSC. For this purpose, a scratch was performed across a monolayer of endothelial cells HMEC-1, and cells were incubated in the presence of CM from treated-iPSC-MSC for 24 h. Then, the percentage of endothelial wound repair was measured. Figure 5A shows that the presence of CM from LPS + Stx-treated iPSC-MSC reduced wound closure compared with nontreated iPSC-MSC CM. Conditioned media from LPS and Stx alone–treated iPSC-MSC did not affect this function. Moreover, when a tubulogenesis assay was performed using HMEC-1, none of the CM affected the formation of new tubes on endothelial cells (Fig. 5B). The addition of Px to control group did not show significant statistical differences compared with control group alone. These results indicate that the effect of Stx and LPS treatment on iPSC-MSC does not favor the repair of endothelial damage.

**Analysis of proteins secreted by iPSC-MSC treated with LPS and or Stx**

With the objective to explore the proteins secreted by iPSC-MSC in the CM after the treatments with LPS and/or Stx, we performed a proteomic analysis, as this technique allows for the simultaneous identification of the proteins present in any given sample, providing a useful and fast method to assess relevant pathways or BPs. Thus, we studied the expression levels of the proteins secreted to the CM by untreated cells (control) or cells treated with LPS, Stx and LPS + Stx iPSC-MSC. Hierarchical clustering of protein abundance data produced four different groups revealing specific secretion profiles associated with each experimental condition (Supplemental Digital Content 2A, http://links.lww.com/SHK/B676). Functional analysis on clustered data resulted in a set of overrepresented ontological terms (Supplemental Digital Content 2B, http://links.lww.com/SHK/B676), in the form of BPs, that exposed important aspects of bacterial toxin treatment.

Mapped protein IDs obtained in CM from treated iPSC-MSC were matched to gene names in databases to access gene ontology.

![FIG. 4. LPS + Stx increased in iPSC-MSC the adhesion to gelatin. Adhered iPSC-MSC to gelatin after 24 h of being treated with LPS and/or Stx. Results were expressed as mean ± S.E.M. n = 4 per group; **P < 0.01.](image)

**FIG. 5. LPS + Stx decreased in iPSC-MSC repair properties in endothelial cells.** Conditioned media from iPSC-MSC treated with LPS and/or Stx were added to endothelial cells for (A) wound healing assay at 18-h postscratch (percentage of endothelial wound repair was measured and representative microphotographs are shown). (B) tubulogenesis assay (percentage of endothelial wound repair was measured and representative microphotographs are shown). Results were expressed as mean ± S.E.M. n = 8 per group; *P < 0.05.
Conditioned media gene ontology over representation analysis obtained from clustered data showed that some proteins are more represented in the CM from iPSC-MSC after treatment with LPS when compared with control cells or with Stx and LPS + Stx treatments. The proteins found in the CM from iPSC-MSC treated with LPS but not with the combination of both toxins are related to BPs like “acute inflammatory response,” “platelet degranulation,” “regulation of fibrinolysis,” and “extracellular matrix organization” (e.g., serpin family H member 1 [SERPINE1], alpha-2-HS-glycoprotein 1 [AHSG1], fibronectin [FN], thrombospondin 1 [THBS1], plasminogen [PLG], pentraxin 3 [PTX3], and cellular communication network factor 2 [CCN2]; Fig. 6A), in line with results obtained in Fig. 2 where LPS polarized iPSC-MSC to a proinflammatory profile increasing migration and adhesion to ECM (Fig. 3 and 4).

Furthermore, LPS + Stx increased the expression of proteins related to BP “IL-12 mediated signaling pathway,” “endopeptidase activity,” and “actin filament organization” (e.g., peptidylprolyl isomerase A [PPIA]) (Fig. 6B). These proteins can be associated with Fig. 5, where a decreased capacity of wound closure was observed in endothelial cells incubated with the iPSC-MS treated with LPS + Stx.

**DISCUSSION**

Mesenchymal stem cells are known to respond and participate in releasing a wide range of cytokines to the environment to modulate tissue damage, often present because of infections (8). In this sense, host immune cells can recognize bacterial toxins and release pro-inflammatory cytokines that can contribute to restore homeostasis (24). Taking this into account, we investigated the effect of two toxins present in HUS, like LPS and/or Stx on iPSC-MSC, speculating that, the repair properties of these cells, may ameliorate endothelial damage.

We use iPSC-MSC as a source of MSCs. It is known that obtaining iPSC-MSC often involves reprogramming fibroblasts to pluripotent stem cells and then a subsequent differentiation to MSCs. This method requires hard work techniques, but when thinking about therapeutic protocols, it is necessary to consider to obtain cells at low cost, in a reproducible way and in large quantities. Most of these requirements are not very compatible with extracting MSCs from each patient, as is painful, invasive, requires a lot of time and is not very reproducible (21,22). Hence, pluripotent derived MSCs arise as a suitable alternative for...
MSC production (23–25) and protocols for obtaining MSCs from iPSC have been improved during several years (22,26,27). For these reasons, we chose to use iPSC-MSC instead of adult MSC in our work.

To the best of our knowledge, this is the first report describing expression of GB3 in iPSC-MSC making this result relevant for featuring iPSC-MSC as direct potential cellular targets for Stx. Moreover, we observed that iPSC-MSC cultured with LPS increased their capacity to migrate and adhere to ECM proteins. These functional results (Fig. 3 and 4) were consistent with the secreted proteins from the CM of iPSC-MSC treated with LPS. For example, SERPINE1 is involved in acute inflammatory responses, AHSG1 is a protein with chronic diseases such as endotoxemia and sepsis (28), THBS1 represents a potent pro-inflammatory signal for macrophages (29), PLG is an enzyme with a crucial role in inflammation (30), FN is a ubiquitous and essential component of the ECM (31), and PTX3 is a prototypic soluble pattern recognition receptor, expressed at sites of inflammation. Systemic levels of PTX3 increase in many (but not all) immune-mediated inflammatory conditions (32).

Although we did not observe an increase in the percentage of wound repair in endothelial cells exposed to CM from iPSC-MSC treated with LPS + Stx, the combination of both toxins, far from repairing, decreased the capacity of iPSC-MSC to restore the endothelial damage and also did not modify the mechanism of new tube formation, pointing iPSC-MSC as another pathophysiological factor that can contribute to endothelial damage in HUS. We hypothesize that the treatments with LPS + Stx on iPSC-MSC induce the release of some factors that decrease the capacity of endothelial cells to repair a wound. In this sense, in the proteomic analysis, we found that the use of LPS + Stx in iPSC-MSC induced the release of proteins related to the BP of “IL-12 mediated signaling pathway” such as PPIA, which is reported to promote apoptosis in endothelial cells (33).

The results observed in this work help understand the role of iPSC-MSC in tissue regeneration, indicating that the combination of both bacterial toxin generated by these cells in response to a particular bacterial toxin should be taken into account. In addition, the fact that the CM from iPSC-MSC could not restore endothelial damage caused by LPS and/or Stx, indicates that the use of iPSC-MSCs as a strategy to regenerate damaged tissues (as is used for other pathologies with tissues injuries), would not be an appropriate option.

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