Therapeutic Effect of Mesenchymal Stem Cell on Organ Ischemia-Reperfusion Injury

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Abstract

The shortage of donor organs is a major global concern. Organ failure requires the transplantation of functional organs. Organ donors are preserved in warm or cold ischemia. Ischemia and reperfusion damage the organs, due to the lack of oxygen during the ischemia step and the oxidative stress during the reperfusion step. Different methodologies were developed to prevent or diminish the level of injuries. Preservation solutions were first developed, followed by the addition of chemical compounds. In addition of inhibitors of mitogen activated protein kinase, inhibitors of the proteasome, mesenchymal stem cells started to be used 13 years ago to prevent or diminish the organ’s injuries. Mesenchymal stem cells (e.g bone marrow stem cells, adipose derived stem cells) have proven to be powerful tools in repairing damaged organs. This review will focus on the use of some bone marrow stem cells, adipose derived stem cells and umbilical cord stem cells on preventing or decreasing the injuries due to ischemia-reperfusion.

Keywords: Ischemia-Reperfusion Injury, Mesenchymal Stem Cells, Treatment.

Abbreviation:

ADSC  Adipose-Derived Stem Cells
ALP   Alkaline phosphatase
ALT   Alanine Aminotransferase
AQP2  Aquaporin 2
AST   Aspartate AminoTransferase
ATP   Adenosine Triphosphate
Bad   Bcl-2-associated death
Bax   BCL2 Associated X, Apoptosis Regulator
Bcl-2  B-Cell CLL/Lymphoma 2
BMSC  Bone Marrow Stem Cells
CCL4  Carbon tetrachloride
CKMB  Creatine kinase-muscle/brain
CXCL2 Chemokine ligand 2
FGF   Fibroblast Growth Factor
G-CSF  Granulocyte-colony stimulating factor
GSH   Glutathione
HGF   Hepatocyte Growth Factor
HIF1a  Hypoxia-inducible factor
HMOX-1 Heme oxygenase (decycling) 1
HO-1  Heme oxygenase-1
I-CAM1 Intercellular Adhesion Molecule 1
IGFBP3 Insulin-like growth factor-binding protein 3
IFN   Interferon
IRI   Ischemia-Reperfusion Injury
**Introduction**

Joseph Murray and David Hume performed the first organ transplantation in 1954 [1]. The USA has 26 donors per millions of people, but Spain has the highest in the world with 35.3 donors per millions of people [2-4] (https://www.pbs.org/newshour/health/country-highest-organ-donation-rates). Even if those numbers seem impressive, there is still an organ donor shortage all over the world. Organ preservation was developed to overcome this problem, but it has been a Herculean task. Ischemia occurs when the organs are taken from the organ donors. The etymology of the word ischemia is from the Greek: iskhein which means restrict and emia for blood. The absence of blood flow leads to the decrease of nutrients supplies to the organs, followed by the disorganization of the cells first, and by consequence of the organs. The final step is the organ failure, conducting to death.

The absence of oxygen supply during the ischemia has a snowball effect. The first step is the depletion and the drastic decrease of the adenosine triphosphate (ATP) production level in the cell. ATP is a major compound for cell survival, controlling the majority of the physiological mechanism of the cells: decrease of glucose production, decrease of the activity of ATP Dependent pumps (Na⁺/K⁺ pump), decrease of the 26S proteasome activity, release of the Ca²⁺...
from the endoplasmic reticulum, decrease of protein synthesis. This last event leads to the decrease of the levels and production of antioxidant enzyme. During the reperfusion, the O₂ influx induces an oxidative stress. The consequence of the oxidative stress is an accumulation of damaged proteins (carbonylated proteins), accumulation of reactive oxidative species (ROS), peroxidation of membrane phospholipids, DNA oxidation (8-hydroxy-2’-deoxyguanosine), etc.[5]

The 3 majors’ organ injuries due to ischemia-reperfusion are: inflammation, oxidative stress and apoptosis [6-8]. To protect the organs from injuries due to the ischemia-reperfusion, preservation solutions were developed over the years to improve the outcome of the transplant, in cold or warm conditions [9-12]. This is a non-exhaustive list of the preservation solution: EuroCollins, Institut Georges Lopez-1 (IGL-1), University of Wisconsin (UW), Celsior, Histidine-tryptophan-ketoglutarate (Custodiol HTK), Belzer’s MPS, Kidney Perfusion Solution (KSP-1)[13-15]. In addition to the solution preservation, chemical compounds were added to improve the efficacy of the preservation solutions, such as inhibitors of the proteasome [16], inhibitors of Mitogen Activated Protein Kinase (MAPK) [17], sodium nitrite [18]. The goal of this review is not to talk about the injuries, but the development of new methodologies to reduce the level of injury.

Mesenchymal stem cells (MSC) are pluripotent stem cells, with the potential to differentiate in different type of cells such as adipocyte, chondrocyte, osteoblast, hepatocyte, and myoblast [19]. For the past 13 years, MSC were used as a biological cellular approach to reduce the injuries due to the ischemia reperfusion of organs. In 2005, the first use of stem cells was mentioned to reduce the ischemia-reperfusion injury in pigs [20]. The authors injected endothelial progenitors’ cells to reduce the size of myocardial infarct and to reduce injuries due to the ischemia-reperfusion. Many others publication followed, showing the potential and protective effect on the injuries. Mesenchymal stem cells have different ways to protect the organs from injury, such as mitochondrial transfer, microvesicles and paracrine effect. The goal of this review is to summarize the protective effect of the bone marrow stem cells, the adipose stem cells and umbilical stem cells and their potential use and development.

1. **Bone Marrow Stem Cells**

Bone marrow stem cells (BMSC) were discovered by Till and McCulloch and used for bone marrow transplantation [21, 22]. Beside the bone marrow transplantation, bone marrow stem cells were studied to be differentiated in different type of cells such as osteoblasts, adipocytes, hepatocytes, myocytes [23]. This opened the door for a multitude of clinical application in the field of regenerative medicine, to treat damaged organs.
One of the bone marrow stem cells capacity is their anti-inflammatory properties [24]. Indeed, it is well known that BMSC can produces cytokines, controlling inflammatory response. BMSC produces IL-10 [25], an important cytokine supporting MSC self-renewal [26]. IL-10 was also reported to be an important anti-inflammatory response [27, 28]. IL-6 is also produced by BMSC, at different level depending on the surface of BMSC surface contact [29]. However, the function of IL-6 is not clear [30]. IL-6 can be pro and anti-inflammatory, depending if IL-6 interacts with membrane IL-6R or with the soluble IL-6R respectively [30]. Trombospodin-2 is also expressed by BMSC and act as an anti-inflammatory cytokine [31, 32]. Others cytokines have only pro-inflammatory activity such as MCP-1 [33], VEGF [34] and FGF [35]. It is important to keep in mind that BMSC produce anti and pro-inflammatory cytokines, and their ratio leads to a pro- or anti-inflammatory activity for the BMSC.

BMSC also have antioxidant properties, playing a major role preventing/decreasing the ischemia-reperfusion injury (IRI). It was shown by Ayatollahi et al. that BMSC blocks the oxidative damage on the liver, caused by the injection of carbon tetrachloride [36]. Injected BMSC protected the rat skin against skin damages and oxidative stress due to D-galactose [37]. Also, BSNC improved the antioxidant defense of aging rats [38]. Other diseases involving oxidative stress, such as arthritis, were treated with success by BMSC in animal models. BMSC were able to decrease the oxidative stress by increasing the levels of GSH, in a rheumatoid arthritis rat model [39]. Other studies reported indirectly the decrease of the oxidative stress. In a rat model, damaging the liver with CCl₄ decreased the levels of lipid peroxidation after the injection of BMSC [36], but the mechanism of action was not studied. Oxidative stress is a major factor accelerating cells death, by increasing the cell damage: oxidation of DNA, of membrane, and of proteins. Estrada showed that stem cell can be used to treat oxidative stress, due to their strong antioxidant properties [40]. Estrada suggests that these properties involve the human telomerase reverse transcriptase. This observation was supported by Brandl et al. which showed that mesenchymal stem cells are more resistant to oxidative stress than others cells (fibroblast and chondrocytes) [41]. It is unclear how the oldest MSC became progressively less resistant to the oxidative stress, but proteins such as Telomeric Repeat Factor 1/2, XRCC5, p21, SIRT1 are controlling this resistance. In the conclusion, we will develop the important of the MSC age in the treatment of the IRI.

Apoptosis is known to occur during the ischemia-reperfusion injury [42], impairing the normal organ physiological function. Intramyocardial injection of bone marrow stem cells in a diabetic cardiomyopathic animal model decreased the level of apoptosis, by increasing the Bcl-2/Bax ratio and by inhibiting the level of activated caspase 3 [43]. Shologu et al. increased the viability of cells grown in hypoxia conditions, by treating them with bone-marrow stem cells [44]. It is unclear how BMSC control the apoptosis, but it was reported that BSMC regulated the activity
of NFkB and the mitochondrial apoptotic pathway [45]. This effect is mediated via a paracrine effect, by factors produced and released by the BMSC [45, 46].

The capability of the BMSC to produce anti-inflammatory cytokines, having anti-apoptotic and antioxidant properties lead laboratories to be interested in BMSC, to decrease ischemia-reperfusion injuries. Many animal studies were conducted to determine if BMSC could decrease injuries due to the ischemia-reperfusion. In 2013, Mostafa Sadek studied the effect of BMSC, and performed an intravenous injection of the BMSC in an acute kidney injury rat model [47]. The injection of the BMSC, after the in vivo ischemia, reduced the level of creatine and serum urea levels, which are signs of kidneys malfunction [48]. In addition, injected BMSC protected the glomeruli from being damaged [47]. The effects of the BMSC are not only positive on the organs with IR but also in connected organs. For example, Dr El-Tahawy induced an IR on rat kidneys, inducing not only kidney injuries but also inducing liver damage. The authors injected the BMSC in an artery and observed a decrease of liver damage through the decrease of the alanine aminotransferase (ALT), aspartate aminotransferase (AST), malondialdehyde (MDA). Inflammation was also reduced (decreased of TNFα levels), and increase of antioxidant proteins such as GSH [49]. In both cases, BMSC were injected in different a blood vessel, but injection site might affect the treatment outcome. Jang et al showed that the injection of BMSC in the artery had a higher positive impact on the renal IR, than injected in the kidney or in the veins [50]. The injection in the artery reduced the serum creatine and the blood urea nitrogen levels, at a high dose of BMSC (4x10⁶ Cells), compared to lower doses (1-2x10⁶ cells).

Not only does the injection of the BMSC help to protect the kidneys from IRI, but supplementary treatment can also improve their efficacy. For example, the pretreatment of the BMSC with melatonin improved the curative effect of BMSC on a renal IR, compared to untreated BMSC. Melatonin treated BMSC increased the expression of antioxidant enzyme (Catalase, SOD-1) and the proliferation cells in kidney, and decreased of the apoptosis [51]. The function of the kidney improved, through the decrease of urea and creatine in the serum. This effect seems to be due to paracrine function through the increase of b-FGF and HGF proteins production of the BMSC. Other publications showed that the combination of melatonin with mesenchymal stem cells improve their curative properties [52, 53]. Another methodology is to activate BMSC with a low-level laser directly targeted to the bone marrow, in a natural way. Activated BMSC migrated from bone marrow to the kidney and decreased the apoptosis, the levels of cystatin C, serum creatine levels and blood urea nitrogen [54, 55]. Those results show that the organs are closely related and connected, and those data are very useful in case of surgeries on patients, especially on heart.

Other organs were treated with BMSC, during IR. Injection of BMSC in a mouse model of liver IR, decreased the inflammatory reaction (decrease of II-6, IL1α, II1β, IL5, IL7, II10, II12, IFNγ),
and decreased the macrophages [56, 57]. In addition to the decreasing apoptosis levels, the authors noticed also a decrease in the reactive oxidative species (ROS) and serum creatine. This correlates with the fact that organs are interconnected and the IR on one organ can affect the activity of others [49]. In this case, liver IR increased the production of creatine. Another study, performed on a rat model, indicates that injection of BMSC decreased liver damage and inflammation (decrease of neutrophil and IL2, IL4, IL6, IL10, CXCL2). Levels of ALT and AST liver markers damage were also decreased [58]. They reported also a decrease of the expression of pro-apoptotic proteins such as caspase 3, bad and Fas.

Lungs were also treated with BMSC cells. In cold ischemia conditions, direct injection of billion BMSC in the left lobe decreased the level of inflammation, by decreasing macrophage, neutrophils, eosinophil and lymphocyte infiltration. Also, less cells were detected in the bronchoalveolar lavage fluid the lungs, indicating a decreased of tissue damage due to the IRI [59]. However, additional information showed that the autophagy levels were increased to compensate the decrease of the proteasome activity, less apoptosis. Other studies showed that BMSC, injected by intravenous injection (IV), can reduce edema and the pulmonary microvascular permeability. The inflammation was decreased (TNFα, IL1β, IL-6) and the expression of anti-inflammatory cytokines, such as IL-10, was increased [60]. It was well demonstrated that cells have an effect by protecting the organs from IRI, but the exosomes or the factors released by the cells can also play a major role in the protection of the organs IRI. Primary rat alveolar cells were cultured in presence of conditioned culture media, containing the factors released by the BMSC. The addition of culture media from BMSC or ADSC, on these alveolar culture cells, decreased the levels of proinflammatory mediators such as IL-10 and decreased the apoptosis levels by blockings the p38 MAPK pathway and increasing Bcl-2 expression [44]. BMSC were also used on rat model for spinal cord IRI [61]. To increase the curative properties of the BMSC, cells were cultured in hypoxia conditions and injected directly in the spinal cord. BMSC protected the spinal cord from IRI, maybe because of the higher expression of HIF1α in the BMSC. This protein controls the expression of genes involved in the angiogenesis, iron metabolism, glucose metabolism and cell proliferation/survival [62].

Table 1 is a summary of the studies reporting the use of bone marrow stem cells, in preventing ischemia-reperfusion injury on major organs (kidney, liver, lung, heart).

<table>
<thead>
<tr>
<th>Targeted Organ/Reference</th>
<th>Animal Model/Human Study</th>
<th>Cells per Dose / Administration/Location</th>
<th>Length of the study</th>
<th>Parameters Reported after Stem Cells Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney [47]</td>
<td>Rat</td>
<td>Unknown/1/ intravenous</td>
<td>1-3 days</td>
<td>Decrease of serum urea level</td>
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<td>Decrease of serum creatinine level</td>
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<td>Decrease of renal cortex damage</td>
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<td>Increase of cell proliferation</td>
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</tbody>
</table>

Table 1 Bone Marrow Stem Cells
<p>| Kidney [49] | Rat | 2.10^6/ 1 time/ intravenous | 5 days (Significant at 5 days) | Decrease of serum urea level Decrease of serum creatinine level Decrease of serum AST/ALT level Decrease of malondialdehyde levels Decrease of TNFα Increase of GSH Less tissue damages |
| Kidney [50] | Rat | 1x10^5/1/ Renal arterial, Renal parenchymal, Tail venous | 14 days | Decrease of serum urea level Decrease of serum creatinine level Protection of glomerular filtration rate 4x10^6 Cells injection via the renal artery provide the best protection |
| Kidney [51] | Rat | 1.10^5/ 3 times BMSC pretreated with melatonin/ Renal parenchymal | Up to 2 months | Decrease of serum urea level Decrease of serum creatinine level Increase of blood vessel Increase of renal cell proliferation Increase of tube formation and proximal tubule cells Increase of b-FGF and HGF expression |
| Kidney [54] | Rat | Laser activation of the bone marrow/ N/A | 4 days | Decrease of serum urea level Decrease of serum creatinine level Decrease of cystatin C Decrease of kidney damage Decrease of inflammation Decrease of apoptosis |
| Kidney [63] | Human Study (135 patients) | 2.10^6 Cells per 1 kg/ 1 time/ Unknown | Long term Follow up | No significant effect was reported |
| Liver [56] | Mouse | 1.10^6/ 1 time/ subcutaneous, intraperitoneal, intravenous or per oral | 6 hours | Decrease of serum AST/ALT/ALP Decrease of serum urea level Decrease of serum creatinine level Decrease of liver injury Decrease of apoptosis/necrosis Decrease of inflammation Decrease of the ROS Increase of NLRP12 and CXCL1 |
| Liver [58] | Rat | 3.10^6/ 1 time/ Portal Vein | 14 days | Decrease of serum AST/ALT Decrease of apoptosis Decrease of liver injury Decrease of inflammation |
| Liver [64] | Rat | 1.10^6/ 1 time/ Portal Vein | 7 days | Decrease of apoptosis/necrosis Increase of liver regeneration |
| Liver [65] | Rat | 3.10^6/ 1 time/ Tail Vein | 5 days | Decrease of serum AST/ALT/LDH Decrease of liver injury |
| Liver [66] | Rat | 1.10^6/ 1 time/ Portal Vein | 24 hours | Decrease of liver injury Decrease of apoptosis Decrease of MDA Decrease of the inflammation Increase of antioxidant enzymes Increase of liver regeneration Increase of the survival |
| Liver [67] | Rat | 1.10^6/ 1 time/ Portal Vein | 3 months | Decrease of liver injury |
| Lung[68] | Rat | 15.10^6/ 1 time/ Overexpressing IL-10/ Penile Vein | 7 days | Decrease of microvascular permeability Decrease of lung injury score Decrease of inflammation Decrease of cells in the bronchoalveolar lavage Decrease of apoptosis Increase of blood oxygenation Recovery of lung weight |</p>
<table>
<thead>
<tr>
<th>Organ</th>
<th>Species</th>
<th>Treatment</th>
<th>Duration</th>
<th>Method</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>Rat</td>
<td>$1.10^6$/1 time/ Penile Vein</td>
<td>Unknown</td>
<td>Decrease of malondialdehyde levels&lt;br&gt;Decrease of myeloperoxidase&lt;br&gt;Decrease of inflammation&lt;br&gt;Decrease of lung injury&lt;br&gt;Decrease of apoptosis&lt;br&gt;Increase of IL-10&lt;br&gt;Increase of SOD&lt;br&gt;Increase of blood oxygenation&lt;br&gt;Recovery of lung weight</td>
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<tr>
<td>Lung</td>
<td>Pig</td>
<td>$5.10^6$ Human Cells/ 1 time/ Pulmonary Artery</td>
<td>4 hours</td>
<td>Decrease of oxygenation&lt;br&gt;Decrease of pulmonary-vascular-resistance&lt;br&gt;Increase of dynamic-lung-compliance</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>Human Study (4 patients)</td>
<td>10.10$^6$/1 time/ Intrabronchial Injection</td>
<td>N/A</td>
<td>Decrease of the inflammation&lt;br&gt;Decrease of macrophage, neutrophils, eosinophil and lymphocyte infiltration&lt;br&gt;Decrease of cells in the bronchoalveolar lavage fluid&lt;br&gt;Decrease of pro-inflammatory cytokines&lt;br&gt;Increase of anti-inflammatory cytokines</td>
<td></td>
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<tr>
<td>Lung</td>
<td>Mice</td>
<td>$5.10^6$/1 time/ Tail Vein</td>
<td>2 hours</td>
<td>Decrease of lung injury&lt;br&gt;Decrease wet/dry ratio&lt;br&gt;Decrease of cells in the bronchoalveolar lavage fluid&lt;br&gt;Decrease of inflammation&lt;br&gt;Decrease of the autophagy&lt;br&gt;Decrease of pAKT&lt;br&gt;Activation of Autophagy</td>
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<tr>
<td>Lung</td>
<td>Rat</td>
<td>$1.10^6$ / 1 time / Penile Vein</td>
<td>2 hours</td>
<td>Decrease of pulmonary microvascular permeability&lt;br&gt;Decrease of lung injuries&lt;br&gt;Decrease of inflammation</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>Rat</td>
<td>200 µl Preconditioned culture media</td>
<td>4 hours</td>
<td>Decrease of lung permeability&lt;br&gt;Decrease of pro-inflammatory cytokines&lt;br&gt;Decrease of neutrophil infiltration&lt;br&gt;Decrease lung injury&lt;br&gt;Increase of macrophages and lymphocyte infiltration&lt;br&gt;Increase of infiltration of M2-macrophages</td>
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<tr>
<td>Lung</td>
<td>Rat</td>
<td>$1.10^6$ (over-expressing HGF)/ 1 time / Penile Vein</td>
<td>24 hours</td>
<td>(all data refers to BMSC injection. The results are improved with BMSC expressing HGF)&lt;br&gt;Curative effect of BMSC increased by HGF&lt;br&gt;Decrease of wet/dry tissue&lt;br&gt;Decrease of lung injury&lt;br&gt;Decrease of apoptosis&lt;br&gt;Decrease of malondialdehyde levels&lt;br&gt;Decrease of myeloperoxidase (MPO)&lt;br&gt;Decrease of CO2 pressure&lt;br&gt;Increase of O2 pressure&lt;br&gt;Increase of SOD levels</td>
<td></td>
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<tr>
<td>Heart</td>
<td>Swine</td>
<td>1.10$^6$ Cardiac Stem Cells+ 2.10$^6$ BMSC Cells/ 1 time / In the Infarct Area</td>
<td>6 weeks</td>
<td>Reduction of myocardial infarction&lt;br&gt;Increase of left ventricular chamber compliance and contractility&lt;br&gt;Restore ejection fraction</td>
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<tr>
<td>Heart</td>
<td>Mouse</td>
<td>$1.10^6$ / 1 time/Coronary Injection before Ischemia</td>
<td>75 min</td>
<td>BMSC (pretreated or not with LPS) increased myocardial function&lt;br&gt;Cardiac function recovery was better with pretreated LPS BMSC compared to untreated BMSC (via Myd88 and STAT3)</td>
<td></td>
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<tr>
<td>Heart</td>
<td>Mouse</td>
<td>$5.10^6$/1 time/Intravenously</td>
<td>6-8 weeks</td>
<td>Increase of c-kit+ BMSC cells in the peripheral blood&lt;br&gt;Increase of VEGF levels&lt;br&gt;No apparent protection from IRI</td>
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<tr>
<td>Heart</td>
<td>Mouse</td>
<td>$1.10^6$ / 1 time/Coronary Injection before Ischemia</td>
<td>24 hours</td>
<td>Reduction of circulating troponin levels&lt;br&gt;Reduction of proinflammatory cytokines (TNFα and IL-18)</td>
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<tr>
<td>Heart</td>
<td>Rabbit</td>
<td>$4.10^6$/1 time/</td>
<td>20 days</td>
<td>Minimal neovascularisation</td>
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</tbody>
</table>
2. Adipose Derived Stem Cells

In 2001, adipose-derived stem cells (ADSC) were described for the first time [80]. As expected, those cells were able to differentiate into the 3 type of germ layers: ectoderm, mesoderm and endoderm [81]. ADSC cells were isolated from liposuction and showed a great potential in the field of regenerative medicine.

Many publications report the anti-inflammatory activity of the ADSC [82]. Injection of ADSC, in a mouse model of bleomycin-induced lung injury, decreased the level of inflammation and fibrosis in the lungs. The ADSC anti-inflammatory activity was due to the inhibition of the production of IL-12 and TNFα, 2 major inflammatory cytokines [83, 84]. Another interesting aspect of this publication is that ADSC induced the macrophage apoptosis, when it was reported that ADSC have an anti-apoptotic property [85]. Anti-apoptotic effect will be discussed later, in this paragraph. ADSC can regulate the inflammatory response by controlling the secretion or by secreting anti-inflammatory molecules. For example, IL-6 is produced by the ADSC and can decrease the inflammation [30, 86]. Under hypoxia and inflammatory conditions, ADSC increased the IL-6 protein production to stimulate cardiomyocyte proliferation [87]. However, IL-6 is an interesting cytokine that can be pro or anti-inflammatory cytokines, depending on the presence of IL-6R in the extracellular environment [30]. In a type 2 diabetic model rat, injection of ADSC regulates the production of inflammatory cytokines such as increasing the production of IL-10, but decreased the production of IL-6, considered as a pro-inflammatory cytokines in this case [88]. Based on the dual role of IL-6 in inflammatory reaction, it will be a bit difficult to consider IL-6 as a marker of anti-inflammatory action. IL-6R should be studied to understand if IL-6 has a pro or anti-inflammatory role. IL-10 is another important anti-inflammatory cytokine that ADSC can produce [89, 90]. An in vitro study showed that the culture of monocytes with LPS induced an inflammatory reaction from the monocytes [91]. The addition of the conditioned culture media of ADSC was sufficient to drastically decrease the levels of inflammation: decrease of TNFα levels and NFκB DNA binding, and by increasing the production of IL-10 and TGFβ [91].

In addition to these important traits, ADSC plays a major role in protecting the cells from apoptosis [92]. The mechanism of action is not fully understood but ADSC blocks the apoptosis by controlling the expression and the activation of specific proteins such as caspase-3, Bad, Bax,
Bcl-2, ... Injection of ADSC directly in lung fibrosis rat model, was followed by the decrease of activated caspase-3 levels, a well-known protein activating cell apoptosis [93-95]. ADSC increased Bcl-2/Bax ratio, positively regulating the cell survival, because these proteins control the mitochondrial membrane potential. If the ratio decreases, the cell is undergoing in apoptosis [96]. Altogether, these results lead to the decrease of apoptosis and the potential protection of the tissue during IRI. Other parameters were studied and showed that ADSC can decrease of oxidative stress and the levels of myeloperoxidase [91], which are markers of IRI.

The first publication mentioning the use of ADSC to protect IRI was published in 2009, on a skin flap model [97]. Application of the ADSC on the skin flap location resulted in the differentiation of the ADSC into endothelial cells, and also in the production of protective growth factors such as VEGF, TGFβ (anti-inflammatory cytokine [98]) and FGF, compared to the skin flap controls [97]. Injection of ADSC into an acute kidney injury (AKI) rat model decreased cell mortality, and reduced kidney damage, shown with the decrease of creatine secretion. The inflammation response was decreased also, by reducing the level of CXCl2 and IL-6 [99]. In this case, IL-6 was involved in the inflammatory response rather than having an anti-inflammatory activity. Many others studies reported similar results by treating different organs such as liver [100-103], kidneys [104], lungs [105], neuro system [106, 107] and heart [108], and they are reported in the Table 2.

Another part of the ADSC cells that was used to treat IRI was the secretome. Any cells secrete proteins and factor in the culture media, and ADSC are not an exception. Secretome has a paracrine and autocrine effect, which carry the curative ADSC compounds. The content of the secretome is composed or proteins, cytokines and RNA [109]. A non-exhaustive list of the secretome compounds is pro-angiogenic factors (FGF, VEGF...), neurotrophic factors (HGF, NGF, BDGF...), cytokines (G-CSF, TNF, PDGF...), adipokines (TNFα, IL-6, IL-8...). The use of secretome demonstrated that the secretome compounds decreased the IRI. For example, the injection of 1 million cells or their secretome had a similar effect on liver IRI. The authors observed decreased the liver damage, less inflammation (I-CAM1, PECAM-1, IL-6), demonstrating that soluble factors secreted by the ADSC carry the curative ADSC properties [101].

Table 2 is a summary of the studies reporting the use of adipose-derived stem cells, in preventing ischemia-reperfusion injury on major organs (kidney, liver, lung, heart).

<table>
<thead>
<tr>
<th>Table 2 Adipose-Derived Stem Cells</th>
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<tbody>
<tr>
<td><strong>Targeted Organ</strong></td>
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<tr>
<td>Kidney [99]</td>
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| Kidney   | Rat     | 1x10^6 /1 time/ in Kidney Cortex                                                   | Up to 14 days | - Decrease of creatinine  
- Decrease of malondialdehyde  
- Decrease of tissue injury  
- Increase of creatinine clearance |
| Kidney   | Cat     | 2x10^6 of ADSC or BMSC or Fibroblasts/1 time/Intra-parenchymal                      | 6 days   | No improvement  |
| Kidney   | Rat     | 1.2 × 10^6 ADSC ± Exendin-4/1 time/intravenous                                     | Up to 3 days | Extendin-4 and ADSC had positive effect on the kidney. Their combination amplified their effect.  
- Decrease of creatinine  
- Decrease of blood urine nitrogen  
- Decrease of urine proteins  
- Decrease of inflammatory cells  
- Decrease of kidney injury  
- Increase of survival  
- Increase of angiogenesis  
- Decrease of apoptosis |
| Kidney   | Rat     | 4x10^6 of ADSC or adipose stromal vascular fraction /1 time/ in Penil vein          |          | - Improve cell proliferation (higher level for SVF)  
- Decrease of apoptosis  
- Decrease of inflammatory cytokines  
- Decrease of tissue injury |
| Liver    | Rat     | 1.2 × 10^6 /1 time/ Tail Vein                                                     | 72 hours | - Decrease of liver damage,  
- Decrease of inflammation  
- Decrease of oxidative stress  
- Decrease of apoptosis |
| Liver    | Mouse   | 1-2 × 10^6 /1 time/ Tail Vein                                                     | Up to 24 hours | - Decrease of ALT  
- Decrease of inflammation  
- Decrease of liver injury  
- Increase of cell proliferation  
- Increase of survival |
| Liver    | Mouse   | 1× 10^6 or exosomes from 1× 10^6 ADSC/1 time/ Tail Vein                           | 24 hours | - Decrease of inflammation  
- Decrease of congestion, necrosis and vacuolization  
- Decrease of liver injury  
- Decrease of neutrophil infiltration |
| Liver    | Rat     | 4× 10^6 /1 time/ Applied on Liver Lobe                                            | Up to 16 days | - Decrease of necrosis  
- Decrease of ALT  
- Decrease of liver injury  
- Increase of proliferation |
| Liver    | Bama miniature pigs | 1×10^5 kg / 1 time / liver parenchyma                                          | Up to 7 days | - All the difference occurs at 1 day (not 7 days after injection)  
- Decrease of AST and ALT  
- Decrease of bilirubin production  
- Decrease of circulating lactate dehydrogenase  
- Decrease of alkaline phosphatase  
- Decrease of malondialdehyde  
- Increase of antioxidant enzymes Levels |
| **Lung [105]** | Rat | $1.5\times 10^6$ /3 times/ penile vein | 72 hours | - Restore O$_2$ levels in the lung’s arteria  
- Decrease of lung injury  
- Decrease inflammation (cytokines, I-CAM1)  
- Decrease of oxidative stress  
- Decrease of apoptosis  
- Increase of antioxidant markers |
| **Lung [123]** | Rat | $1.2\times 10^6$ ADSC (pretreated with melatonin)/1 time/ penile vein | 72 hours | - Restore arterial O$_2$ pressure  
- Decrease of lung injury  
- Decrease of inflammatory cytokines  
- Decrease of cell infiltration  
- Increase alveoli number  
- Increase of anti-oxidative enzyme |
| **Lung [124]** | Rat | $1.2\times 10^6$ ADSC (serum deprived, healthy or combined) /1 time/ penile vein | 96 hours | Results from combined have a better outcome than the others:  
- Restore blood oxygen saturation  
- Restore right ventricular systolic pressure  
- Decrease of lung injury  
- Decrease of inflammation  
- Decrease of apoptosis  
- Decrease of cell infiltration  
- Decrease of bronchioles hemorrhages  
- Increase of number of alveoli  
- Increase of antioxidant Enzyme |
| **Heart [108]** | Mouse | $1\times 10^6$ ADSC or $1\times 10^6$ ADSC (overexpressing HMOX-1) /1 time/ subcutaneously | 1 hour | - Improvement of left ventricular end-diastolic pressure and left ventricular diastolic pressure  
- Increase of anti- and pro-inflammatory cytokines  
- Increase of cardio-protective proteins expression  
- Decrease of infarct size (ADSC+HMOX-1)  
- No change or heart rate and coronary flow |
| **Heart [125]** | Rat | $4\times 10^6$ ADSC (pretreated or not) /1 time/ Two peri-infarct regions and one was within the infarct area | 28 days | Pretreated ADSC with curcumin had a better effect than naïve ADSC  
- Improvement of ejection fraction, fractional shortening, end-systolic volume, end-diastolic volume  
- Increase of ADSC attachment to the heart  
- Increase of blood vessel and VEGF levels  
- Decrease of infarction size  
- Decrease of apoptosis |
| **Heart [126]** | Rat | $400 \mu g$ of ADSC exosome /1 time/ Tail vein Injection | 3 hours | - Decrease of infarct size  
- Decrease of LDH, creatine kinase-muscle/brain (CKMB), cardiac troponin I serum levels  
- Decrease of apoptosis  
- Increase of Beta-Catenin and wnt3a proteins |
3. **Umbilical Cord:**

In 1994, umbilical cord mesenchymal stem cells (UCMSC) were discovered and characterized [127]. Even if the first study reports that the UCMSC don’t seem to be able to differentiate in different type of cells, another group reported that UCMSC could differentiate into osteoblast-like cells [128]. UCMSC are capable to have an anti-inflammatory activity. Yoo et al showed that UCMSC anti-inflammatory properties are similar to the others mesenchymal stem cells such as bone-marrow stem cells, adipose-derived stem cells [129]. UCMSC decreased the levels of secreted IFNγ and TNFα from T-Cell. Niemann-Pick type C is a rare disease, caused by mutations on the gene NPC1 [130]. The disease affects peripheral organs but also the central nervous system, mainly through a pro-inflammatory reaction. In a Niemann-Pick type C mouse model, injected UCMSC decreased the level of inflammation, through the increase of IL-10 secretion and by reducing the astrocyte activation [131]. As mentioned previously, it is possible that the UCMSC could modulate the activity of the astrocyte via their secretome. This was partially proven with the co-culture of UCMSC and CD14(+) monocytes. UCMSC decreased the secretion of IFNγ by CD14(+) and their proliferation. This effect was due to the secretion of prostaglandin E2 (PGE2), produced by UCMSC. PGE2 is produced continuously by the UCMSC, but in this experiment, it was reported that IL-1β, secreted by the CD14(+) cells, induced the production of PGE2 as a negative feedback loop [132]. Other neurogenerative diseases, involving inflammation, were treated with success in animal models [133]. Other factors than PGE2 are involved in the anti-inflammatory activity of the UCMSC: PTX3, IGFBP3, TIMP-1 [134, 135]. TSG-6 can interact with PTX3, to downregulate the inflammation. Transplantation of UCMSC in mice acute lung injury decreased the inflammatory activity in the lungs (decrease of TNFα, MIP-2, IFNγ). In parallel to the decrease of the pro-inflammatory cytokines, the UCMSC stimulated the secretion of IL-10, as it was reported in the BMSC and ADSC [136].

In a spinal cord injury rat model, inflammation increased in the injured area. Increased IL-1β levels, in the injured area, were downregulated by the injection of UCMSC. It was reported that UCMSC secreted high levels of anti-inflammatory cytokines IL-10 [137]. Inflammation observed in others organs were downregulated by the injection of UCMSC: heart [138], arthritis [139], kidney [140], gastro-intestinal tract [141], lungs [142].

As many others mesenchymal stem cells, umbilical cord stem cells protect the cells from the apoptosis. The first paper reporting the anti-apoptotic effect of the umbilical cord stem cells was published in 2008. SH-SY5Y cells, cultured in hypoxic conditions entered in apoptosis [143]. After 3 days of hypoxic conditions, SHY-SYSY cells were at 85% in apoptosis, but for the SH-SYSY treated with umbilical cord cells, the percentage of apoptosis dropped at 7%. The levels of apoptosis were detected with the decrease of cleaved caspase-3 and Poly (ADP-ribose) polymerase (PARP) levels, and by detecting the levels of annexin V [143]. C2C12 cells cultured in a serum-starvation condition are dying by apoptosis. The high levels of cleaved PARP was one of the apoptotic causes. However, the coculture of the C2C12 with UCMSC decreased the level of apoptosis, by paracrine effect via the secretion of XCL1. The addition of XCL1 in the serum-starvation condition was enough to decrease the level of apoptosis. This is an
important example showing the potential curative effect of stem cells secretome from through their paracrine effect [144]. It is not necessary to inject directly the cells, which can be a problem if their growth is not controlled. Their uncontrolled migration can lead to the ectopic growth and impairment of organ function. In a cartilage damage mouse model, the injection of monosodium iodoacetate induced cell apoptosis in the cartilage [145]. To block or decrease cell apoptosis, the authors injected umbilical cord stem cells, in the articulation. UCMSC decreased the level of apoptosis (decreased of cleaved caspase 3). The reduction of the apoptosis level in the cartilage helped to regenerate the cartilage [145].

Progressive death of retinal ganglion cells can be one cause leading to visual impairment. One approach to treat the disease is to protect the retinal ganglion from death, by using different approaches and one of them is the use of umbilical cord stem cells [146]. Injection of UCMSC in the limbus reduced and delayed the level of apoptosis, without stopping it. In fact, the level of apoptosis was not different after 28 days of treatment with the disease eye. The UCMSC could only delay the death of retinal ganglion, without preventing it. It is not always the case. Diabetic rats, treated with UCMSC, had lower level of apoptosis (increase of Bcl-2, decrease of activated caspase 3). The authors found that the anti-apoptotic is mediated by the production and release of IGF-1 by the UCMSC, and through the activation of Akt kinase [147]. IGF-1 is an important hormone controlling the cell proliferation of the pancreatic cells, which concurs with the results obtained by Zhou’s group [148].

Increase of the oxidation during the reperfusion step is a major cause of organ injury. It is for this reason the antioxidant properties of compounds used to decrease the IRI is important. As other mesenchymal stem cells, umbilical cord mesenchymal stem cells have antioxidant properties [36, 39, 149]. Injection of UCMSC in mice, with a chronic liver injury (due to CCL4 injection), decreased the level of injury due to oxidation: decrease of malondialdehyde and increase of antioxidant enzyme levels (SOD and GSH). The liver injury score was reduced by the injection of the UCMSC in the liver [150].

Table 3 is a summary of the studies reporting the use of umbilical cord stem cells, in preventing ischemia-reperfusion injury on major organs (kidney, liver, lung, heart).

<table>
<thead>
<tr>
<th>Targeted Organ</th>
<th>Animal Model/Human Study</th>
<th>Cells per Dose / Administration/Location</th>
<th>Length of the study</th>
<th>Parameters Reported due to Stem Cells Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney [151]</td>
<td>Rat</td>
<td>1x10⁶ /1 time/ Left Carotid Artery</td>
<td>72 hours</td>
<td>- Decrease of creatinine</td>
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<td>- Decrease of blood urea nitrogen</td>
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<td>- Decrease of apoptosis</td>
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<td>- Decrease of inflammation</td>
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<td>- Decrease of kidney Injury</td>
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<td>- Increase of cell proliferation</td>
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<tr>
<td>Kidney [152]</td>
<td>Mouse</td>
<td>1x10⁶ /1 time/ Intravenous</td>
<td>Up to 72 hours</td>
<td>- Increase of creatinine</td>
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<td>- Increase of blood urea nitrogen</td>
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<td>- Increase of K⁺ and PO₄⁻</td>
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<td>- Increase of apoptosis</td>
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<td>- Increase of kidney injury</td>
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<td></td>
<td>- Increase of myeloperoxidase activity</td>
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<td>(even in lungs, but not on liver and heart)</td>
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<td></td>
<td>- Increase of inflammation</td>
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<td>- Increase of neutrophil infiltration</td>
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</tbody>
</table>
| Kidney [153] | Mouse | $2 \times 10^6 / 1$ time/ Caudal Vein | 5 days | - Decrease of creatinine
- Decrease of blood urea nitrogen
- Decrease of injury
- Decrease of apoptosis
- Decrease of macrophage infiltration
- Decrease of monocyte chemoattractant protein 1
- Increase of proliferation

| Kidney [154] | Mouse | $5 \times 10^3 / 1$ time/ Renal artery | 7 days | - Decrease of creatinine
- Decrease of blood urea nitrogen
- Decrease of renal injury
- Decrease of reactive oxidative species
- Decrease of macrophage infiltration
- Decrease of neutrophil infiltration
- Decrease of kidney fibrosis
- Faster increase of microvascular density
- Early protective effect against apoptosis

| Kidney [155] | Mouse | $1 \times 10^6 / 1$ time/ Intraperitoneally | 2 days | - Decrease of creatinine
- Decrease of blood urea nitrogen
- Decrease of tubule damage
- Decrease of inflammatory cytokines
- Increase of anti-inflammatory cytokines
- Increase of VEGF (VEGF controls the curative effect)
- No dose effect of the numbers of cells (up to $3 \times 10^6$).

| Kidney [156] | Rat | $1 \times 10^6 / 1$ time/ Intraperitoneally | Up to 49 days | - Faster decrease of blood urea nitrogen
- Decrease of creatinine
- Decrease of blood urea nitrogen
- Decrease of macrophage infiltration
- Decrease of inflammation
- Decrease of fractional excretion of sodium
- Decrease of HO-1 expression
- Decrease of miR29a and miR34a expression
- Decrease of p21 expression
- Increase of aquaporin2 protein expression
- Increase of creatine clearance
- Increase of Klotho expression
- Increase of MnSOD expression
- Same results reported for the 7 days follow up except for:
  - Creatine clearance, kidney injury,
- Same results reported for the 49 days study, except for: No changes in AQP2
<table>
<thead>
<tr>
<th>Organ</th>
<th>Species</th>
<th>Treatment</th>
<th>Route</th>
<th>Duration</th>
<th>Findings</th>
</tr>
</thead>
</table>
| Kidney | [157] | Rat | $1 \times 10^6$ /1 time / Tail vein | 30 days | - Decrease of kidney damage  
- Decrease of creatinine plasma levels  
- Decrease of urea levels  
- Decrease or urine albumin levels  
- Decrease of uric acid  
- Decrease of malondialdehyde  
- Increase of GSH, GST and catalase levels |
| Kidney | [158] | Human study for allograft | $2 \times 10^6$ per kilogram before graft 1 time, vein injection / and $5 \times 10^6$ per during surgery, renal arterial injection | 1 Year follow up | End points (Results not reported yet NCT02490020):  
- Allograft rejection  
- Kidney function  
- Post-operatives’ complications  
- Infection, pneumonia, bleeding. |
| Liver | [159] | Rat | $3 \times 10^6$ (in 2D or 3D structure)/1 time/ in intraperitoneal | 48 hours | The 3D structure has faster effect than 2D structure, for the following comments:  
- Decrease of liver injury  
- Decrease of plasma level of ALT and AST  
- Decrease of plasma total bilirubin  
- Faster liver Regeneration  
- Decrease of apoptosis (2D structure has no effect)  
- Increase of IL-6 and TNFα |
| Liver | [160] | Rat | Extracellular vesicles from cells/ 1 time/ intraperitoneal | 24 hours | Extracellular vesicles have a better positive effect than cell injection.  
Protective effect of cells mediated by MnSOD.  
- Decrease of ALT  
- Decrease of AST  
- Decrease of ALP  
- Decrease of apoptosis  
- Decrease of neutrophils infiltration  
- Decrease of IL-6, II-1β and TNFα  
- Decrease of Reactive Oxygen Species and 8-isoprostane  
- Decrease of Myeloperoxidase activity  
- Decrease of Malondialdehyde  
- Increase of Antioxidant enzyme production |
| Liver | [161] | Mouse | $1 \times 10^6$ Cells /$1 \times 10^6$ Cells Treated Rapamycin / $1 \times 10^6$ Cells Treated Rapamycin+AMD3100 / $1 \times 10^6$ Cells Treated Rapamycin+3-MA 1 time/ in a peripheral vein | 24 hours | Effect increased by Rapamycin treatment, compare to MSC alone (Blocked by AMD3100 and 3-MA)  
- Decrease of ALT and AST.  
- Decrease of liver injury and apoptosis  
- Decrease of Neutrophil Infiltration  
- Decrease of 8-isoprostane  
- Decrease of IL-6, II-1β and TNFα |
<p>| Lung | [162] | Rat | $1 \times 10^5$ Cells or $1 \times 10^6$ Cells overexpressing ACE-2/1 | 24 hours | Over-expression of ACE-2 improve MSC |</p>
<table>
<thead>
<tr>
<th>Organ</th>
<th>Species</th>
<th>Treatment</th>
<th>Time</th>
<th>Protective Effect</th>
</tr>
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</table>
| Lung  | Rat     | 1x10⁶ Cells or 1x10⁶ ADSC overexpressing ACE-2/1 | 72 hours | - Decrease of inflammatory cytokines levels  
- Decrease of Protein Carbonyl level  
- Decrease of Apoptosis  
- Decrease of DNA damage  
- Increase of antioxidative levels
| Lung  | Mouse   | 3x10⁶ Cells or extracellular vesicles (EV) from 3x10⁶ Cells /1 time/ perfusion | 2 hours | Cells or EV injection had similar effect. Restore airways resistance, pulmonary pressure  
- Decrease of lung weight  
- Decrease of neutrophils infiltration  
- Decrease of myeloperoxidase levels  
- Decrease of pro-inflammatory cytokines  
- Decrease of macrophage and NKT activation
| Heart | Rats    | 5x10⁵ Cells treated with oxytocin 1 day (C1ox) or 7 days (C7ox) /1 time/ injected into the peri-infarct area | Up to 4 weeks | - Expression of cardiac troponin T, 7 days after injection.  
- Higher expression of cardiac troponin T, Cnx43 and α-sarcomeric actin, in C7ox compared to C1ox  
- Increase of ejection fraction in C7ox group.  
- Decrease of fibrosis

**Conclusion and Perspectives**

Mesenchymal stem cells were discovered long time ago, but we are just starting to understand and use their incredible curative potential to improve the patient health. Our knowledge is still partial about their curative properties, but the increase of the studies reporting results about the mesenchymal stem cells will help to accelerate the time for translational application. We are at the dawn of testing the potential of stem cells in preventing or decreasing the injuries on organs after ischemia. This review reported only the treatment of lungs, heart, kidneys and liver with MSC or the MSC’s secretome, but many others organs were tested such as intestine [166], limbs [167], retina [168], spinal cords [169].
This review compiled publications showing the positive impact of mesenchymal stem cells to prevent or decrease IRI, on major organs. Other organs (brain, intestine,...) were also treated with success, in animal models [170, 171]. For all the studies mentioned above, whatever type of cells the authors are using, the authors are usually analyzing the same parameters in the same organs. For example, in the liver IR, the authors usually verify the level of AST and ALT in the blood, or creatinine for the kidneys IR. These markers were currently used to determine the organ injuries in clinics [172, 173]. Combination of markers were used for the detection of cancers to increase the detection sensitivity [174, 175]. Based on a similar approach, new markers in combination with the classic markers mentioned in this review for IR could be used to confirm the curative/protective effect of the injected MSC. A recent publication shown that miRNA can be used to determine the level of organ injury [176]. miRNAs are small RNA (19-24 nucleotides in length) discovered in 1993 by Dr Victor Ambros [177]. These miRNA bind complementary RNA to disturb mRNA translation (as a review [178]), giving to the miRNA the power to control any cellular activity. miRNA 122 is the major miRNA expressed in the liver, under the control of HNF4α [179], and was one of the first miRNA described in the liver [180]. miRNA 122 is involved in regulating Hepatitis C virus replication cycle, in hepatocyte differentiation, in the cholesterol and fatty acid synthesis and in the hepatocellular carcinoma formation [181]. In the case of disease or liver injury, miRNA 122 is released in the blood circulation, which is used to determine liver injuries such as AST and ALT [181]. Hepatic ischemia/reperfusion is a major clinical problem for liver transplantation. Cell apoptosis occurs in the liver ischemia-reperfusion, followed by the release of the miRNA122 [182]. By itself, miRNA 122 levels detected in the blood are already very sensitive to indicate the liver injury level [183, 184]. The level of apoptosis was correlated with the circulating levels of AST, ALT and miRNA122, making miRNA122 a marker that can be used in combination with others [182]. The levels of liver enzymes (AST, ALT, ALP) decreased also, in parallel of the serum levels of miRNA 122 and miRNA-34a. Histopathological evaluation of liver injuries showed that the treatment protected the liver from IRI [185]. Other miRNA were reported to be related with organs injury and could be used as marker: miRNA 34a, miRNA-223, miRNA-370, miRNA-155 for the liver, miRNA-15, miRNA-17, miRNA-99/100 for the heart, miRNA-21 for the kidney, miRNA146a, miRNA155 for the lungs [176]. miRNA can be used as at target to decrease the level of organ injury. Let's use the apoptosis as an example, that can be transposable to any other mechanism inducing organ IRI. During IRI, ROS levels are increased and activated miRNA such as miRNA-1, miRNA-29, miRNA-133 and miRNA-21. miRNA-1 and miRNA-29 are pro-apoptotic when miRNA-21 and miRNA-133 are anti-apoptotic [186]. A strategy could be to inject anti-apoptotic miRNA in preservation solution to over-balance the effect of the pro-apoptotic miRNA. It’s an approach that was used for the past 10 years when miRNA are injected directly in animal models to treat diseases such as cancer [187-189]. To develop such strategies, accurate knowledge of miRNA targets and function is necessary. As mentioned above, miRNA-1 is a pro-apoptotic miRNA [190] but it can be also anti-apoptotic [191]. In addition of increasing the levels or anti-apoptotic miRNA by delivery in the organ, injected miRNA can also decrease the levels of ROS which is the major cause of the apoptosis in IR [192]. miRNA can be used to decrease the level of injury after IR, and should be combined with others strategies.

In some cases, the injection of the MSC had no effect [63, 119], or even worsened the injuries due to the ischemia-reperfusion [152]. The reasons that could explain the failure of these treatment could be due to different factors. The first reason could be the number of injected MSC, insufficient to treat the
organs. Rodrigues et al injected 1x10^6 cells in a rat (average weight 250 gr), meaning 4x10^6 cells per kg [156], or El-Tahawy injected 11x10^6 cells per kg in a rat albinos [49]. In the other hands, in the clinical trials, patients were injected 2x10^6 cells per kg [63, 159], which represents 18% to 50% of the number of cells injected in rats. The number of cells can have an impact in the “pharmacodynamics” that could lead to protecting (partially or totally) the organ from injuries [50]. The second reason is the methodology to inject the cells in the animal. Injecting cells in an organism is the cheapest and easier methodology, but it is a random treatment because the migration of the cells can’t be controlled. In addition, the survival of non-attached cells decreases a lot [193]. Directly grafting the MSC on the organs could be an efficient way not only to increase the number of cells targeted on the organs, but it might also increase the efficacy of the treatment based on Hamdi et al work [194]. Different scaffolds can be considered to graft directly the MSC on the organs: ceramic, collagen, hyaluronan [195] or none [196]. The third important factor is the age of the MSC donors. In undifferentiated ADSC cells, the level of Bax, caspase-9, Cyt-c and, caspase 3 increased over time [197]. A review reports that in vivo aging MSC showed a decreased proliferation potential, decreased differentiation potential, a decreased telomerase length and an increase of genetic instability [198]. It can also speculate that aged MSC could have a different and less efficient secretome, than younger MSC, meaning their capability to cure or treat the damaged organ. It was reported that ADSC from younger donors might be more effective in treating cells, tissues etc. [199-201]. All these reported data revealed important information about the choice of MSC donors and the potential outcome in treating patients with them.

In conclusion, because of the immunocompetences, MSC could be banked in large quantity and used when it is necessary [202, 203], in different ways (injection, cell sheets, 3D printing, to decrease/prevents the IRI and overcome the shortage of organs or help in surgeries. They can be used in combination with compounds or miRNA.

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**Conflicts of Interest:** The author is employee of Emmaus Medical, Inc. (Torrance, CA, USA).
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Figures
Figure 1: Effect of MSC on organs Ischemia-Reperfusion Injury
Figure 2: Actual and Potential Improvement of treatment of IRI with MSC