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

Joan Oliva [1,2].

1 Emmaus Medical, Inc., Torrance, 90503, CA, USA

2 Department of Medicine, LA BioMed at Harbor UCLA Medical Center, Torrance, 90502, California, U.S.A.

Send Correspondence to:

Joan Oliva, Ph.D

Department of Medicine

Division of Hematology

LA Biomed

1124 W. Carson St.

Torrance, CA 90502

Tel 310-214-0065 / 424-571-7639

Fax 310-214-0075

Email: joliva@emmauslifesciences.com

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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 AmnioTransferase
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
CCL_4	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

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LDH	Lactate dehydrogenase
LPS	Lipopolysaccharides
MAPK	Mitogen Activated Protein Kinase
MDA	Malondialdehyde
miRNAs	micro-RNA
MnSOD	Manganese Superoxide Dismutase
NKT	Natural Killer T
NLRP12	NLR Family Pyrin Domain Containing 12
Nrf2	Nuclear factor erythroid 2-related factor 2
PDGF	Platelet-derived growth factor
PECAM-1	Platelet endothelial cell adhesion molecule
PGE2	Prostaglandin E2
PTX3	Pentraxin 3
SOD	Super Oxide Dismutase
MSC	Mesenchymal Stem Cells
NFkB	Nuclear Factor kappa-light-chain-enhancer of activated B cells
PARP	Poly (ADP-ribose) polymerase
SIRT	NAD-dependent deacetylase sirtuin
TIMP-1	Metalloproteinase inhibitor 1
TNF	Tumor Necrosis Factor
UCMSC	Umbilical Cord Mesenchymal Stem Cells
VEGF	Vascular endothelial growth factor
XCl1	X-C Motif Chemokine Ligand 1

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 Ca^{2+}

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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 O_2 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.

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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, BSMC 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 CCl_4 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 importantance 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

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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 ($4x10^6$ Cells), compared to lower doses ($1\text{-}2x10^6$ 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 Il-6, IL1α, Il1β, IL5, IL7, Il10, Il12, IFNγ),

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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 1 Bone Marrow Stem Cells

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

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Kidney [49]	Rat	2.10^5/ 1 time/ intravenous	5 days (Significant at 5 days)	Decrease of serum urea level Decrease of serum creatinine level Decrease of serum AST/ALT Decrease of malondialdehyde levels Decrease of TNFα Increase of GSH Less tissue damages
Kidney [50]	Rat	1-4x10^6/1/ 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	1.10^6/ 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	3.10^5/ 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

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Lung [69]	Rat	1.10^6/ 1 time/ Penile Vein	Unknown	Decrease of malondialdehyde levels Decrease of myeloperoxidase Decrease of inflammation Decrease of lung injury Decrease of apoptosis Increase of IL-10 Increase of SOD Increase of blood oxygenation Recovery of lung weight
Lung [70]	Pig	50.10^6 Human Cells/ 1 time/ Pulmonary Artery	4 hours	Decrease of oxygenation Decrease of pulmonary-vascular-resistance Increase of dynamic-lung-compliance
Lung [59]	Human Study (4 patients)	$10. 10^7$/ 1 time/ Intrabronchial Injection	N/A	Decrease of the inflammation Decrease of macrophage, neutrophils, eosinophil and lymphocyte infiltration Decrease of cells in the bronchoalveolar lavage fluid Decrease of pro-inflammatory cytokines Increase of anti-inflammatory cytokines
Lung [71]	Mice	5.10^5/ 1 time/ Tail Vein	2 hours	Decrease of lung injury Decrease wet/dry ratio Decrease of cells in the bronchoalveolar lavage fluid Decrease of inflammation Decrease of the autophagy Decrease of pAKT Activation of Autophagy
Lung [60]	Rat	1.10^6 / 1 time / Penile Vein	2 hours	Decrease of pulmonary microvascular permeability Decrease of lung injuries Decrease of inflammation
Lung [72]	Rat	200 µl Preconditioned culture media	4 hours	Decrease of lung permeability Decrease of pro-inflammatory cytokines Decrease of neutrophil infiltration Decrease lung injury Increase of macrophages and lymphocyte infiltration Increase of infiltration of M2-macrophages
Lung [73]	Rat	1.10^6 (over-expressing HGF)/ 1 time / Penile Vein	24 hours	(all data refers to BMSC injection. The results are improved with BMSC expressing HGF) Curative effect of BSMC increased by HGF Decrease of wet/dry tissue Decrease of lung injury Decrease of apoptosis Decrease of malondialdehyde levels Decrease of myeloperoxidase (MPO) Decrease of CO_2 pressure Increase of O_2 pressure Increase of SOD levels
Heart [74]	Swine	1.10^6 Cardiac Stem Cells+ 2.10^8 BMSC Cells/ 1 time / In the Infarct Area	6 weeks	Reduction of myocardial infarction Increase of left ventricular chamber compliance and contractility Restore ejection fraction
Heart [75]	Mouse	1.10^6 /1 time/Coronary Injection before Ischemia	75 min	BMSC (pretreated or not with LPS) increased myocardial function Cardiac function recovery was better with pretreated LPS BMSC compared to untreated BMSC (via Myd88 and STAT3)
Heart [76]	Mouse	5.10^6 /1 time/Intravenously	6-8 weeks	Increase of c-kit+ BMSC cells in the peripheral blood Increase of VEGF levels No apparent protection from IRI
Heart [77]	Mouse	1.10^5 /1 time/Coronary Injection before Ischemia	24 hours	Reduction of circulating troponin levels Reduction of proinflammatory cytokines (TNFα and IL-18)
Heart [78]	Rabbit	4.10^6 /1 time/	20 days	Minimal neovascularisation

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		intramuscularly or intravenously		No Inflammation
Heart [79]	Swine	3.10^7 Cells (Pretreated with Atorvastatin) / 1 time / In the Infarct or Peri-infarct area	4 weeks	Decreased of defect areas Decrease of inflammation Decrease of fibrosis Decrease of apoptosis Increase of ejection fraction

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,

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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 2 Adipose-Derived Stem Cells

Targeted Organ	Animal Model/Human Study	Cells per Dose/ Administration/Location	Length of the study	Parameters Reported due to Stem Cells Treatment
Kidney [99]	Rat	$5x10^6$ /1 time/Intra-Arterial	Up to 72 hours	- Reduction of mortality - Reduction of creatinine - Reduction of intratubular cast formation - Decrease of macrophage Infiltration - Decrease of tubular epithelial cell necrosis

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				- Decrease of inflammation
Kidney [110]	Rat	$1x10^6$ /1 time/Intra-Renal	14 days	- Decrease of blood urea nitrogen - Decrease of creatinine - Decrease of kidney Injury - Decrease of apoptosis - Increase of antioxidant enzyme
Kidney [111]	Mouse	$1x10^5$ /3 times/Tail Vein	14 days	- Decrease of acute tubular necrosis - Decrease of interstitial macrophage infiltration - Decrease of inflammatory response
Kidney [112]	Rat	$1.2x10^6$ ASC (pre or not pretreated with cyclosporin)/1 time/Intra-Renal	72 hours	Cyclosporin amplify the protective properties of ADSC - Decrease of inflammation - Decrease of kidney injury - Decrease of circulating levels of creatinine and blood urea nitrogen - Decrease of apoptosis - Decrease of oxidative stress - Decrease of macrophage infiltration - Decrease of DNA damage - Increase of cell viability - Increase of antioxidant activity - Increase of blood vessel density - Increase of von Willebrand Factor production
Kidney [113]	Rat	$2x10^6$ /1 time/Intra-Renal/ intravenous	24 hours	- Decrease of creatinine - Decrease of kidney Injury - Decrease of apoptosis - Decrease or inflammation
Kidney [114]	Rat	$1x10^6$ /1 time/ in the kidney or intra-renal artery	14 days	- No adverse event - Increase of survival - Improvement of renal function
Kidney [115]	Rat	$1x10^6$ /1 time/ Injection in Kidney cortex 2D or 3D aggregates	24 hours	- 3D aggregates have a better outcome - Decrease the injury - Decrease of apoptosis - Decrease of creatinine - Decrease of blood urea nitrogen
Kidney [116]	Rat	$2x10^6$ /1 time/ in Kidney Cortex	Up to 14 days	- 3D aggregates have a better outcome - Decrease of creatinine - Decrease of blood urea nitrogen - Decrease of apoptosis - Increase of vascularization
Kidney [117]	Rat	Exosome or $1.2x10^6$ ADSC or both/1 time/ in Kidney Cortex	72 hours	- ADSC or exosomes+ADSC have a better outcome than exosome alone. - Decrease of creatinine - Decrease of kidney injury - Decrease of inflammation - Decrease of oxidative stress

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Kidney [118]	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 [119]	Cat	$2x10^6$ of ADSC or BMSC or Fibroblasts/1 time/intra-parenchymal	6 days	No improvement
Kidney [120]	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 [121]	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 [103]	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 [102]	Mouse	$1\text{-}2 \times 10^6$ /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 [101]	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 [100]	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 [122]	Bama miniature pigs	1×10^6 /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

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Lung [105]	Rat	$1.5x10^6$ /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	$1.2x10^6$ 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	$1.2x10^6$ 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	$1x10^6$ ADSC or$1x10^6$ 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	$4x10^6$ 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 µg 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

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				- Activation of GSK3B

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 IFNɣ 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 IFNɣ 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, IFNɣ). 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-SY5Y cells were at 85% in apoptosis, but for the SH-SY5Y 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 grow 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 CCL_4 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 3: Umbilical Cord Stem Cells

Targeted Organ	Animal Model/Human Study	Cells per Dose / Administration/Location	Length of the study	Parameters Reported due to Stem Cells Treatment
Kidney [151]	Rat	$1x10^6$ /1 time/ Left Carotid Artery	72 hours	- Decrease of creatinine - Decrease of blood urea nitrogen - Decrease od apoptosis - Decrease of inflammation - Decrease of kidney Injury - Increase of cell proliferation
Kidney [152]	Mouse	$1x10^6$ /1 time/ Intravenous	Up to 72 hours	- Increase of creatinine - Increase of blood urea nitrogen - Increase of K+ and PO_4^- - Increase of apoptosis - Increase of kidney injury - Increase of myeloperoxidase activity (even in lungs, but not on liver and heart) - Increase of inflammation - Increase of neutrophil infiltration

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				- Decrease of cell proliferation
Kidney [153]	Mouse	$2x10^6$ /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	$5x10^5$ /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	$1x10^6$ /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 $3x10^6$).
Kidney [156]	Rat	$1x10^6$ /1 time/ Intraperitoneally	Up to 49 days	For the 2 days study: - 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

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				expression, kidney damage, increase of Klotho
Kidney [157]	Rat	$1x10^6$ /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	2×10^6 per kilogram before graft 1 time, vein injection / and 5×10^6 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	$3x10^6$ (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, Il-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	$1x10^6$ Cells /$1x10^6$ Cells Treated Rapamycin / $1x10^6$ Cells Treated Rapamycin+AMD3100 / $1x10^6$ 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, Il-1β and TNFα
Lung [162]	Rat	$1x10^6$ Cells or $1x10^6$ Cells overexpressing ACE-2/1	24 hours	Over-expression of ACE-2 improve MSC

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		time/ perfusion		protective effect. - Restore Arterial oxygen saturation - Restore right ventricular systolic blood pressure - Increase of Alveoli number - Increase of endothelial cells - Increase of GPX, GR and $NQO1^+$ - Decrease of lung injury - Decrease of DNA damage - Decrease of Macrophage infiltration
Lung [163]	Rat	$1x10^6$ Cells or $1x10^6$ ADSC overexpressing ACE-2/1 time/ perfusion	72 hours	Over-expression of ACE-2 improve MSC protective effect. - Decrease of inflammatory cytokines levels - Decrease of Protein Carbonyl level - Decrease of Apoptosis - Decrease of DNA damage - Increase of antioxidant levels
Lung [164]	Mouse	$3x10^6$ Cells or extracellular vesicles (EV) from $3x10^6$ 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 [165]	Rats	$5x10^5$ 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 an d 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].

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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 are 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

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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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Figures

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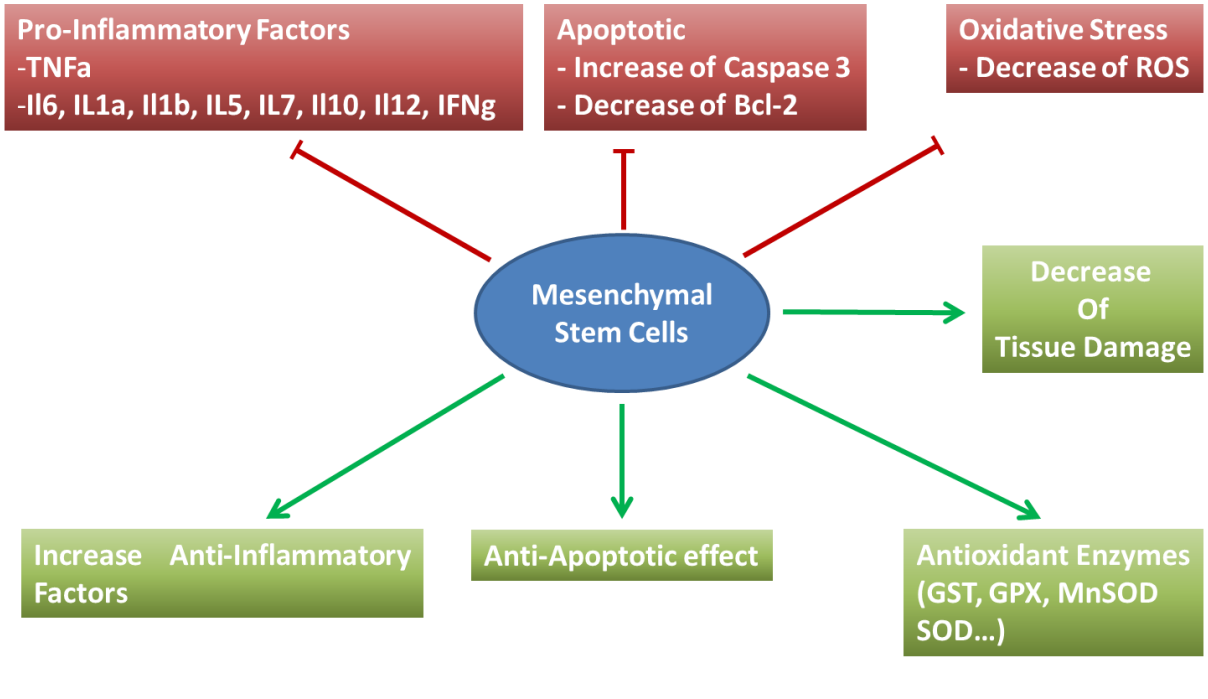

Figure 1: Effect of MSC on organs Ischemia-Reperfusion Injury

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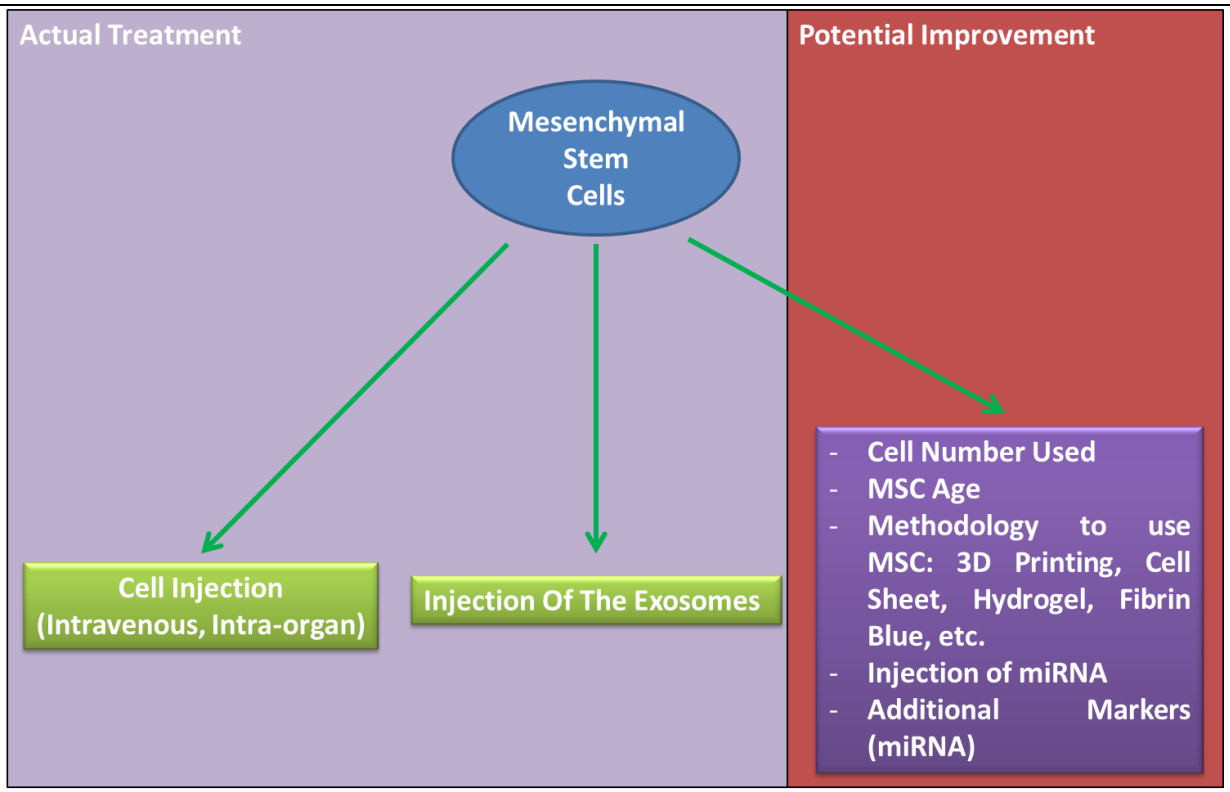

Figure 2: Actual and Potential Improvement of treatment of IRI with MSC