Pre-conditioning Strategies for Mesenchymal Stromal/Stem Cells in Inflammatory Conditions of Livestock Species

Benjamin Uberti¹, Anita Plaza² and Claudio Henríquez³*

¹ Instituto de Ciencias Clínicas, Facultad de Ciencias Veterinarias, Universidad Austral de Chile, Valdivia, Chile, ² Instituto de Patología Animal, Facultad de Ciencias Veterinarias, Universidad Austral de Chile, Valdivia, Chile, ³ Instituto de Farmacología y Morfotecnología, Facultad de Ciencias Veterinarias, Universidad Austral de Chile, Valdivia, Chile

Mesenchymal stem/stromal cells (MSCs) therapy has been a cornerstone of regenerative medicine in humans and animals since their identification in 1968. MSCs can interact and modulate the activity of practically all cellular components of the immune response, either through cell-cell contact or paracrine secretion of soluble mediators, which makes them an attractive alternative to conventional therapies for the treatment of chronic inflammatory and immune-mediated diseases. Many of the mechanisms described as necessary for MSCs to modulate the immune/inflammatory response appear to be dependent on the animal species and source. Although there is evidence demonstrating an in vitro immunomodulatory effect of MSCs, there are disparate results between the beneficial effect of MSCs in preclinical models and their actual use in clinical diseases. This discordance might be due to cells’ limited survival or impaired function in the inflammatory environment after transplantation. This limited efficacy may be due to several factors, including the small amount of MSCs inoculated, MSC administration late in the course of the disease, low MSC survival rates in vivo, cryopreservation and thawing effects, and impaired MSC potency/biological activity. Multiple physical and chemical pre-conditioning strategies can enhance the survival rate and potency of MSCs; this paper focuses on hypoxic conditions, with inflammatory cytokines, or with different pattern recognition receptor ligands. These different pre-conditioning strategies can modify MSCs metabolism, gene expression, proliferation, and survivability after transplantation.

Keywords: mesenchymal stem cells, mesenchymal stromal cells, livestock animals, pre-conditioning, inflammation, hypoxia

INTRODUCTION

Mesenchymal stromal/stem cells (MSCs) have been studied extensively for more than 40 years, with a large body of evidence supporting that MSCs can resolve inflammation and promote tissue repair in various inflammatory conditions, since they can interact with and modulate both innate and adaptive immune responses (1). Many of the mechanisms involved in these processes have been elucidated, but their relevance depending on the cell species and source of the cells remains to be studied, especially in large animals.
The activation of local resident sensor cells of the immune response, responsible for the non-specific detection of microorganisms or tissue damage, leads to the secretion of first-order cytokines that promote the arrival of cells of the adaptive immune response; depending on the inciting cause, local resident sensor cells (epithelial, mast cells, innate lymphoid, and effector memory cells) trigger cellular and humoral effector responses (2). In this sense, the immune and inflammatory response can be modulated at different levels, and MSCs can actively and passively influence both innate and adaptive components of the immune response (3). Recent works in animals deal in-depth with the molecules and pathways that confer MSCs their ability to inhibit lymphocyte proliferation and activation, modify macrophage and lymphocyte polarization, promote cell survival, and therefore modulate inflammation (4–7). However, these mechanisms can be enhanced through different physical and chemical alternatives, which could in turn augment the therapeutic effects of MSCs. The objective of this review is to describe the use of low oxygen tension, pro-inflammatory cytokines or Pattern Recognition Receptors (PRR) as MSCs pre-conditioning mechanisms which could potentially increase their effectiveness and translational potential for inflammatory conditions in livestock animals.

IMPROVING MSCs IMMUNOMODULATORY FUNCTION

Low oxygen (O2) tension, high concentrations of inflammatory cytokines, and even presence of microorganisms, are all hallmarks of an inflamed tissue which play a key role in the metabolism of the cells present at the site of injury, and can modulate their functions. It is in these environments where transplanted MSCs will have to perform, by modulating the inflammatory/immune response and promoting the regenerative process. This has led to exploration of ways to pre-condition MSCs in order to “jumpstart” their physiology and strengthen their therapeutic mechanisms, looking to increase the amount of cells that arrive to the site of inflammation and their survival, while also increasing their anti-inflammatory and regenerative effects (8–10). Thus far, pre-conditioning strategies for MSC oriented therapies in veterinary medicine have focused especially on small animals (11–14). In livestock species, studies on MSCs have mostly been published in the last decade (Table 1), with some of their immunomodulatory mechanisms described (Table 2). Much remains to be determined regarding pre-conditioning strategies for MSCs in livestock.

Hypoxia

The issue of optimal culture conditions for MSCs has been under investigation for many years, and the optimum oxygen tension in which to culture cells is an important consideration. Physiological oxygen tension varies greatly between different tissues and can range from 12% in blood to values below 1% (61). MSCs may experience a variety of oxygen tensions, for instance 1–7% in bone marrow or 10–15% in adipose tissue: higher levels of oxygen may lead to early senescence, oxidative stress, DNA damage and lower proliferation (62–64). Regardless of the value, physiological oxygen tension in tissues is significantly lower than the atmospheric tension of the gas generally used in MSCs cultures.

Hypoxic pre-conditioning greatly modifies MSCs physiology, increasing colony formation unit (CFU) number and survival while limiting apoptosis and senescence, mainly but not exclusively through the Hypoxia-Inducible Factor (HIF)-1α mediated pathway (65–68). Some authors have pointed out that these effects could entail an impairment in differentiation capacity (69, 70), but there are mixed opinions on this topic, since other authors describe that low O2 tension culture conditions preserve and even increase MSCs differentiation ability (71–73). Additionally, hypoxia has been shown to increase MSC migration both in vitro and in vivo, modulating the expression of chemokine receptors and integrins (72, 74–77), and also promoting their vasculogenic potential by increasing expression of VEGF and Angiopoietin (Ang-1) (78–80), both important factors that increase MSCs therapeutic potential. Furthermore, in different pre-clinical models, hypoxic pre-conditioning favors the therapeutic effect of MSCs, enhancing their arrival and engraftment into injured tissue where they promote the survival of tissue resident cells, all of which could be beneficial in clinical settings (76, 81–86). Hypoxic pre-conditioning also enhances MSCs regulation of the inflammatory response by increasing expression of key molecules that modulate the immune response. Hypoxia elevates expression of Indoleamine-pyrrole 2,3-dioxygenase (IDO), HLA-G, Prostaglandin-endoperoxide synthase 2 (PTGS-2), and interleukin (IL)-10 (87–89) and decreases pro-inflammatory molecules such as tumor necrosis factor (TNF)-α, IL-1β and IL-6, and nitric oxide (NO) (87) in different contexts both in vitro and in vivo, all of which modify cellular fate and condition the inflammatory response by decreasing inflammation and promoting a pro-resolutive context through different pathways (90, 91). Authors have also described that hypoxic pre-conditioning modifies MSCs metabolism and may cause metabolic disruption by increasing glucose uptake and usage, leading to lactate accumulation which attenuates T cell division (92). Interestingly, the MSCs expression of the soluble form of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) increases under hypoxic conditions, which could affect the lymphocyte activation process by interacting with co-stimulatory molecules of antigen presenting cells (93). Hypoxic priming of MSCs also modifies T cell polarization, by stimulating regulatory T cell proliferation via paracrine mechanisms (89). It is also important to note that hypoxic culture conditions do not impair MSCs ability to respond to inflammatory stimuli nor their immunosuppressive capacities (94). In fact, HIF-1α expression by MSCs induced by hypoxic culture conditions increases their modulatory capacities (95): experimental overexpression of HIF-1α, mimicking hypoxia, has been shown to enhance MSC immunomodulatory properties, while its silencing decreases their immunosuppressive potential (95–98).

There is little information regarding the effect of low O2 tension culture conditions upon MSCs physiology in livestock animals, and mixed results have been reported. In bovines, a
### TABLE 1 | Effects of mesenchymal stromal/stem cells (MSCs) on inflammatory conditions in livestock animals.

| Animal species | Study design | Origin | Disease | Effect | References |
|----------------|--------------|--------|---------|--------|------------|
| Bovine         | in vivo      | AT-MSC | Mastitis| Safety of transplantation. No difference in SCC. Decreased CFU in MSC-treated quarters in an experimental *S. aureus*-induced mastitis model | (15) |
|                | in vitro and in vivo | AM-MSC | Mastitis| Decrease in *S. aureus* growth and increase in mammary epithelial cell survival by MSC-CM in vitro. No difference between SCC in quarters treated with CM or antibiotics of animals with acute and chronic mastitis | (16) |
|                | in vitro     | AT-MSC | Fertility| Co-culture of bovine embryos with AT-MSC increases blastocyst development rates and quality when compared to granulosa cells | (17) |
| Ovine          | in vivo      | BM-MSC | ARDS    | Intrapulmonary transplantation of MSC ameliorates airway inflammation induced by LPS, reducing inflammatory cells, proteins, immunoglobulins and inflammatory cytokines in the BALF | (18, 19) |
|                | in vivo      | UC-MSC | Neuroinflammation| MSCs administered IV reduce cerebral inflammation induced with LPS in preterm fetus, by limiting astrogliosis, improving cerebral cell apoptosis, and myeline content | (20–22) |
|                | in vivo      | PB-MSC | Wound healing| Locally transplanted MSCs do not increase wound closure but slightly improve neovascularization and skin inflammation | (23) |
|                | in vivo      | BM-MSC | Tendonitis| Locally transplanted MSCs do not increase wound closure | (24) |
|                | in vivo      | BM-MSC | Arthritis| MSCs administered IV reduce clinical score and modulate inflammation in a model of arthritis | (25, 26) |
|                | in vivo      | UC-MSC | Cerebral hypoxia/ischemia| MSCs reduce brain inflammation limiting white matter injury and accelerating self-repair. MSCs also reduce plasmatic levels of TNFα and increase IL-10 | (21) |
| Caprine        | in vivo      | AT-MSC | Mastitis| Small improvement but no significant difference in fibrosis, inflammatory infiltration and cell proliferation before and after MSC transplantation | (27) |
| Equine         | in vivo      | AT-MSC | Tendonitis| MSCs limit lesion progression, increase organization of collagen fibers, and decrease inflammatory infiltrate. No changes in gene expression | (28) |
|                | in vivo      | CB-MSC | Wound healing| MSCs transplanted IV do not accelerate wound healing, but do decrease both inflammatory and anti-inflammatory cytokine expression within the wound | (29) |
|                | in vivo      | CB-MSC | Wound healing| Locally applied MSCs with PRP improve wound healing | (30) |
|                | ex vivo      | PB-MSC | Skin infection| MSCs reduce viability of methicillin-resistant *S. aureus* biofilms | (31) |
|                | in vivo      | AT-MSC | Laminitis| Locally transplanted MSCs with platelet-rich plasma improve hoof vascularization | (32) |
|                | in vivo      | UC-MSC | Osteoarthritis| Intra articular administration of MSCs significantly improved OA clinical scores. No significant improvement from repeated injections | (33) |
|                | in vivo      | BM-MSC | Osteoarthritis| Improved pain and disability. Additionally, MSC treatment improved cartilage quality | (34, 35) |
|                | ex vivo      | AT-MSC | Uveitis| Reduction of activation and IFN-γ secretion by T CD 4+ cells isolated from horses with recurrent uveitis. Increase in memory cells | (36) |
|                | ex vivo      | AT-MSC | Endometritis| Direct injection of MSCs to endometrial slices induce expression of IL-8 and MMP-9 | (37) |
| Porcine        | in vivo      | BM-MSC | Endometritis| Reduced uterus inflammation induced by sperm challenge | (38) |
|                | in vivo      | BM-MSC | Myocardial infarction| MSCs reduce fibrosis | (39) |
|                | in vivo      | BM-MSC | Myocardial infarction| MSCs improve cardiac function | (39, 40) |
|                | in vivo      | BM-MSC | Myocardial infarction| MSCs improve cardiac function | (41) |
|                | in vivo      | AT-MSC | Renal injury| MSCs and/or their EVs limit renal inflammation, improve oxygenation, fibrosis, renal flow, and glomerular filtration | (42–46) |

AM-MSC, Aminiotic membrane-derived MSC; AT-MSC, adipose tissue-derived MSC; BALF, Broncho-alveolar lavage fluid; BM-MSC, bone marrow-derived MSC; CB-MSC, Cord blood-derived MSC; CFU, Colony forming unit; CM, Conditioned medium; CT, Cord tissue-derived MSC; EV, extracellular vesicles; OA, osteoarthritis; PB-MSC, Peripheral blood-derived MSC; SCC, Somatic cells count; UC-MSC, umbilical cord-derived-MSC.
recent study shows that culture with low oxygen tension may increase BM-MSCs survival and proliferation with limited effect on gene expression, mainly upregulating the expression of genes related to cell stress, growth, and metabolism (99). Similar results were described in buffalo AT-MSCs, in which hypoxic culture conditions (5% O2) enhanced proliferation, colony formation and differentiation potential, increasing expression of HIF-1α and secretion of basic fibroblast growth factor and vascular endothelial growth factor (VEGF) (100). In sheep, hypoxic culture resulted in faster bone marrow (BM)-MSC population doublings per day, and cell colony formation and viability were not significantly affected (101, 102). Although hypoxia enhanced in vitro BM-MSC chondrogenesis (102), this did not translate into increased cartilaginous repair tissue formation following cell transplantation into cartilage defects in vivo (103). While some authors report that hypoxic pre-conditioning of porcine MSCs had no effect on proliferation or cell migration (104, 105), others do describe an increase in cell proliferation and impaired osteogenic differentiation in both BM and adipose tissue (AT)-MSCs under hypoxic conditions (106). These conflicting results could be due to differences between culture protocols; for example, Antebi et al. (79) describe that MSCs proliferate significantly faster during 48 h of culture than during 10 days of culture, in both cases under 1% O2 hypoxic conditions. Additionally, authors mention that porcine MSCs cultured under hypoxia had upregulated expression of VEGF and the anti-inflammatory cytokines IL-1 receptor antagonist (RA) and granulocyte-macrophage colony-stimulating factor (GM-CSF),
with concomitant downregulation of the pro-inflammatory cytokine IL-8 (79).

In horses, MSCs therapy is of special interest in musculoskeletal diseases such as osteoarthritis, tendon and ligament injuries, bone repair, among others (Table 1). Low oxygen tension culture conditions (5%) attenuate the proliferative capacity of equine AT-MSCs but not BM-MSCs; however, in normoxic (21% O2) conditions a greater proportion of cells were in S phase of cellular cycle, indicating that both cell populations were more active (69). Hypoxic culture seems to keep cells more undifferentiated than normoxic culture, and this is supported by a tendency of hypoxic MSCs to increase expression of embryonic markers (69). This is also described by Griffon et al. (70) who found that hypoxic (5%) culture together with chitosan affected cell yield but improved the stemness of UC-MSCs, with increased expression of embryonic markers such as NANOG, OCT4, and SOX2. In an in vitro fracture hematoma model in horses, hypoxic conditions (1% O2) favored survival of MSCs and an increase in osteogenesis, and MSCs survival was correlated with a decrease in live lymphocytes (107).

Hypoxic conditions (1% O2) produce an increase in tenogenic gene expression in rabbit BM-MSC, which correlates with their increased capacity in promoting patellar tendon repair in vivo determined either by both tissue reparation and biomechanical analysis (108). Similar beneficial results are described in a study using hypoxic (1% O2) pre-conditioning in rabbit BM-MSCs used in combination with hyaluronic acid for the treatment of osteoarthritis. Those results show that the addition of hypoxic pre-conditioned BM-MSCs reduce cartilage loss and surface abrasion, with an improvement in histological features compared with hyaluronic acid alone (109). Hypoxic pre-conditioning (1% O2) also enhances the therapeutic effects of rabbit BM-MSC on a disc degeneration model, greatly improving MSC ability to reduce damage and improving extracellular matrix deposition (110).

**Pro-inflammatory Cytokines**

MSCs -and even their culture supernatant- can modulate phagocyte functions in vitro and in vivo without requiring activation (52, 111). However, activation of MSCs with pro-inflammatory cytokines sets in motion several pathways involved in the arrival of MSCs to the injured tissue and is also relevant to development of their full immunomodulatory potential (61). This notion about the need of pro-inflammatory stimuli to activate the immunomodulatory capacity of MSCs is particularly interesting because it could allow identification of the mechanisms required for suppression of the immune response (112). This evidence indicates the potential of MSCs for the treatment of diverse inflammatory conditions in large animals (Table 1).

TNF-α and interferon (IFN)-γ are the most common pro-inflammatory cytokines used for pre-conditioning MSCs, either alone or in combination. IFN-γ is a key modulator of the IDO-Kynurine pathway which has been shown to be a key component of the immunomodulatory arsenal of MSCs and is involved in several of the reported effects on immune cells (3). In this regard, it is logical to find that pre-conditioning MSCs with IFN-γ results in increased expression of IDO, and of other key immunomodulatory molecules such as prostaglandin (PG)E-2, transforming growth factor (TGF)-β, NO, IL-10, tumor necrosis factor-inducible gene 6 protein (TSG-6) and Programmed death-ligand 1 (PD-L1). These molecules can suppress the cytotoxic activity of natural killer (NK) cells, lymphocyte proliferation and cytokine secretion (113–116), and macrophage polarization (116, 117). Additionally, priming MSCs with IFN-γ has been shown to impair a type 3 immune response characterized by cells that produce IL-17 (118). Regarding function of B cells, pre-treatment with IFN-γ increases MSCs’ capacity to reduce B-cell proliferation and immunoglobulin production (119). Some of these effects of IFN-γ are increased with the addition of TNF-α, since the combination of both cytokines increases the expression of immunomodulatory molecules such as IDO, PTGS-2 and inducible nitric oxide synthase (iNOS) (51). This improvement of immunomodulation by MSCs pre-conditioned in vitro with IFN-γ and/or TNF-α has also been observed in vivo when the cells are transplanted into animals in different preclinical models of inflammatory conditions, such as colitis, diabetes, or graft-vs.-host disease (GVHD) (117, 118, 120–123).

One potential complication of pre-conditioning with IFN-γ is that along with the rise of MSCs immunomodulatory ability there is also an increase in immunogenicity: in horses and rabbits, IFN-γ pre-conditioning has been shown to upregulate MHC class I and II (60, 124), which can be avoided by pretreating cells with TGF-β2 (125). Pre-conditioning with IL-17 could be an alternative, since it not only confers MSCs greater immunomodulatory capacities such as suppression of T cell proliferation and activation, inhibition of type 1 response, and increased induction of Treg cell, but also unlike IFN-γ, it does not induce immunogenicity (126).

In swine, stimulation of MSCs with TNF-α or IL-1 increased their migratory capacity although this effect seems to be dependent on the tissue source (127). Additionally, treatment of porcine AT-MSCs with a IFN-γ, TNF-α and IL-6 cytokine cocktail increased the expression of immunomodulatory molecules such as GBP4, IL1-RA, and IDO while impairing pro-inflammatory cytokines IL-6, IL-17, and TNF-α in vitro (128). In horses, MSCs incubation with TNF-α and IFN-γ produces a great increase in the main immunomodulatory paracrine molecules secreted by MSCs such as PTGS-2, IDO, iNOS, and IL-6, while downregulating the expression of other molecules like IL-10, TGF-β1, TSG-6, which are relevant in other species but have no proven role in large animal MSCs immunomodulatory mechanisms (129).

**Pattern Recognition Receptors Ligands**

The innate immune system is the first line of defense against microorganisms and is constantly surveying the body for the presence of pathogen-associated molecular patterns (PAMPs), which are detected by highly conserved receptors known as PRR. One of the main PRR families are Toll-like receptors (TLR), and MSCs preferentially express TLR 1–6 (9, 130). Another important PRR family are the nucleotide-binding oligomerization domain (NOD)-like receptor (NLR), whose activation of NLR proteins results in inflammatory responses mediated either by NF-κB,
MAPK or Caspase-1 activation, accompanied by subsequent secretion of pro-inflammatory cytokines (131). Ligands for both types of PRR have been considered for pre-conditioning of MSCs, since they not only influence their immunomodulatory functions but also promote antibacterial activity.

The effects on MSCs immune modulation will vary depending on which TLR ligand is used. For instance, Fuenzalida et al. (132) compared the stimulation of TLR-3 and TLR-4 by using polyinosinic-polycytidylic acid [poly(I:C)] and lipopolysaccharide (LPS), respectively. They determined that TLR3 ligands produced a stronger immunosuppressive phenotype compared with MSCs preconditioned with TLR4 ligands, since poly(I:C) induces greater IDO expression which correlates with inhibition of lymphocyte proliferation in vitro and improved DSS-induced colitis in vivo. Similar results were described by another study in which pre-conditioning with poly(I:C) improved human MSCs immune modulatory properties, decreasing pro-inflammatory cytokines and increasing systemic IL-10 levels in colonic tissues. This was associated with the inhibition of type 1 and 3 immune responses and promotion of Treg differentiation (133).

In horses, there are a few studies about MSCs pre-conditioning using TLR ligands. Cassano et al. (134) showed that TLR-3 or 4 stimulation in MSCs enhances their ability to suppress mitogen stimulated T cells proliferation, with MHC class II positive MSCs having a stronger immunosuppressive activity than MHC class II negative MSCs. Interestingly, MSCs have also been shown to have antimicrobial activity, both by constitutively secreted factors such as defensins, hepcidin and lipocalins, and indirectly by activation of innate immune effector cells (135). Similarly, equine MSCs constitutively express the antimicrobial lipocalin-2 whose expression is augmented by stimulation with LPS, and which could mediate the limitation of the growth of Escherichia coli by MSCs-conditioned media (55). Furthermore, MSCs stimulation with poly(I:C) and [γ-d-Glu-mDAP (IE-DAP)], an NLR agonist, stimulates antimicrobial peptide production and increases bactericidal activity, also suppressing biofilm formation and enhancing neutrophil bactericidal functions (136).

**CONCLUSION**

Pre-conditioning with hypoxia, pro-inflammatory cytokines, or Pattern Recognition Receptors Ligands seem to enhance MSC survival, arrival, engraftment, proliferation, immunomodulatory or pro-regenerative functions, and may increase their therapeutic efficacy. Despite limitations in their use in veterinary medicine, especially due to cost and high variability between species, culture and cryopreservation protocols, and tissue sources, MSCs seem to be a promising alternative therapy for inflammatory and immune-mediated conditions. It is critical to further our understanding of known and novel mechanisms by which MSCs modulate inflammatory processes in livestock animals.

**AUTHOR CONTRIBUTIONS**

BU: manuscript writing and edition. AP and CH: manuscript writing. All authors contributed to the article and approved the submitted version.

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**REFERENCES**

1. Singer NG, Caplan AI. Mesenchymal stem cells: mechanisms of inflammation. *Anna Rev Pathol.* (2011) 6:457–78. doi: 10.1146/annurev-pathol-011110-130230
2. Ammuniati F, Romagnani C, Romagnani S. The 3 major types of innate and adaptive cell-mediated effector immunity. *J Allergy Clin Immunol.* (2015) 135:626–35. doi: 10.1016/j.jaci.2014.11.001
3. Shi Y, Wang Y, Li Q, Liu K, Hou J, Shao C, et al. Immunoregulatory mechanisms of mesenchymal stem cell and stromal cells in inflammatory diseases. *Nat Rev Nephrol.* (2018) 14:493–507. doi: 10.1038/s41581-018-0023-5
4. Dias IE, Pinto PO, Barros LC, Viegas CA, Dias IR, Carvalho PP. Mesenchymal stem cell therapy in companion animals: useful for immune-mediated diseases? *BMC Vet Res.* (2019) 15:358. doi: 10.1186/s12917-019-2087-2
5. Macdonald ES, Barrett JG. The potential of mesenchymal stem cells to treat systemic inflammation in horses. *Front Vet Sci.* (2019) 6:507. doi: 10.3389/fvets.2019.00507
6. Vogla, Adamic M, Vengust M, Majdic G. Stem cells in veterinary medicine-current state and treatment options. *Front Vet Sci.* (2020) 7:278. doi: 10.3389/fvets.2020.00278
7. Bukowska J, Szostek-Mioduchowska AZ, Kopcewicz M, Walendzik K, Machcinska S, Gawronska-Kozak B. Adipose-derived stromal/stem cells from large animal models: from basic to applied science. *Stem Cell Rev Rep.* (2021) 17:719–38. doi: 10.1007/s12015-020-10049-y
8. Saparov A, Ogay V, Nurgozhin T, Jumabay M, Chen WC. Preconditioning of human mesenchymal stem cells to enhance their regulation of the immune response. *Stem Cells Int.* (2016) 2016:3924858. doi: 10.1155/2016/3924858
9. Najar M, Krayem M, Merimi M, Burny A, Meuleman N, Bron D, et al. Insights into inflammatory priming of mesenchymal stromal cells: functional biological impacts. *Inflamm Res.* (2018) 67:467–77. doi: 10.1007/s00011-018-1131-1
10. Lee BC, Kang KS. Functional enhancement strategies for immunomodulation of mesenchymal stem cells and their therapeutic application. *Stem Cell Res Ther.* (2020) 11:397. doi: 10.1186/s13287-020-01920-3
11. Yu J, Liu XL, Cheng QG, Lu SS, Xu XQ, Zu QQ, et al. G-CSF and hypoxic conditioning improve the proliferation, neural differentiation and migration of canine bone marrow mesenchymal stem cells. *Exp Ther Med.* (2016) 12:1822–8. doi: 10.3892/etm.2016.3553
12. Kim SM, Li Q, An JH, Chae HK, Yang JI, Ryu MO, et al. Enhanced angiogenic activity of dimethyloxalylglycine-treated canine adipose tissue-derived mesenchymal stem cells. *J Vet Med Sci.* (2019) 81:1663–70. doi: 10.1292/jvms.19-0337
of limb wounds following intravenous allogeneic cord blood-derived multipotent mesenchymal stromal cell therapy in horses. Cells. (2021) 10:2972. doi: 10.3390/cells10112972
30. Iacono E, Berlino B, Pirrone A, Antonelli C, Brunori L, Romagnoli N, et al. Effects of mesenchymal stem cells isolated from amniotic fluid and platelet-rich plasma gel on severe decubitus ulcers in a septic neonatal foal. Res Vet Sci. (2012) 93:1439–40. doi: 10.1016/j.rvsc.2012.04.008
31. Marx C, Gardner S, Harman RM, Wagner B, Van De Walle GR. Mesenchymal stromal cell-secreted CCL2 promotes antibacterial defense mechanisms through increased antimicrobial peptide expression in keratinocytes. Stem Cells Transl Med. (2020) 10:1666–79. doi: 10.1002/stcm.21-0058
32. Angelone M, Conti V, Biacca C, Battaglia B, Pecorari L, Piana F, et al. The contribution of adipose tissue-derived mesenchymal stem cells and platelet-rich plasma to the treatment of chronic equine laminitis: a proof of concept. Int J Mol Sci. (2017) 18:2122. doi: 10.3390/ijms18102122
33. Magri C, Schramme M, Febre M, Cauvin E, Labadie F, Saulnier N, et al. Comparison of efficacy and safety of single versus repeated intrathecally injected of allogeneic neonatal mesenchymal stem cells for treatment of osteoarthritis of the metacarpophalangeal/metatarsophalangeal joint in horses: a clinical pilot study. PLoS ONE. (2014) 14:e0221317. doi: 10.1371/journal.pone.0221317
34. Orozco L, Munar A, Soler R, Alberca M, Soler F, Huguet M, et al. Treatment of knee osteoarthritis with autologous mesenchymal stem cells: a pilot study. Transplantation. (2013) 95:1555–61. doi: 10.1097/TP.0b013e3182912ada
35. Vega A, Martin-Ferrero MA, Del Canto F, Alberca M, Garcia V, Munar A, et al. Treatment of knee osteoarthritis with allogeneic bone marrow mesenchymal stem cells: a randomized controlled trial. Transplantation. (2015) 99:1681–90. doi: 10.1097/TP.0000000000001678
36. Saldinger NK, Nelson SG, Bellone RR, Lassaline M, Mack M, Walker NJ, et al. Horses with equine recurrent uveitis have an activated CD4+ T-cell phenotype that can be modulated by mesenchymal stem cells in vitro. Vet Ophthalmol. (2020) 23:160–70. doi: 10.1111/vop.12704
37. Falomo ME, Ferroni L, Tocco I, Gardin C, Zavan B. Immunomodulatory role of adipose-derived stem cells on equine endometriosis. Biomed Res Int. (2015) 2015:141485. doi: 10.1155/2015/141485
38. Ferris RA, Frisbie DD, Mccue PM. Use of mesenchymal stem cells or autologous conditioned serum to modulate the inflammatory response to spermatozoa in mares. Theriogenology. (2014) 82:36–42. doi: 10.1016/j.theriogenology.2014.02.015
39. Amado LC, Saliaris AP, Schuleri KH, St John M, Xie JS, Cattaneo S, et al. Cardiac repair with intramyocardial injection of allogeneic mesenchymal stem cells after myocardial infarction. Proc Natl Acad Sci USA. (2005) 102:11474–9. doi: 10.1073/pnas.0504388102
40. Amado LC, Schuleri KH, Saliaris AP, Boyle AJ, Helm R, Oskouei B, et al. Multimodality noninvasive imaging demonstrates in vivo cardiac regeneration after mesenchymal stem cell therapy. J Am Coll Cardiol. (2006) 48:2116–24. doi: 10.1016/j.jacc.2006.06.073
41. Noort WA, Oerlemans MI, Rozemuller H, Feyen D, Jaksani S, Stecher D, et al. Human versus porcine mesenchymal stromal cells: phenotype, differentiation potential, immunomodulation and cardiac improvement after transplantation. J Cell Mol Med. (2012) 16:1827–39. doi: 10.1111/j.1582-4934.2011.01455.x
42. Elbahmi B, Eirin A, Li Z, Zhu XY, Zhang X, Lerman A, et al. Mesenchymal stem cells improve medullary inflammation and fibrosis after revascularization of swine atherosclerotic renal artery stenosis. PLoS ONE. (2013) 8:e67474. doi: 10.1371/journal.pone.0067474
43. Zhu XY, Uربيeta-Caceres V, Krier JD, Textor SC, Lerman A, Lerman LO. Mesenchymal stem cells and endothelial progenitor cells decrease renal injury in experimental swine renal artery stenosis through different mechanisms. Stem Cells. (2013) 31:117–25. doi: 10.1002/stem.1263
44. Eirin A, Zhang X, Zhu XY, Tang H, Jordan KL, Grande JP, et al. Renal vein cytokine release as an index of renal parenchymal inflammation in chronic experimental renal artery stenosis. Nephrol Dial Transplant. (2014) 29:774–82. doi: 10.1093/ndt/gft305
45. Eirin A, Zhu XY, Puranik AS, Tang H, McGurren KA, Van Wijnen AJ, et al. Mesenchymal stem cell-derived extracellular vesicles attenuate kidney inflammation. Kidney Int. (2017) 92:114–24. doi: 10.1016/j.kint.2016.12.023
46. Zhao Y, Zhu X, Zhang L, Ferguson CM, Song T, Jiang K, et al. Mesenchymal stem/stromal cells and their extracellular vesicle progeny decrease injury in poststomietic swine kidney through different mechanisms. *Stem Cells Dev.* (2020) 29:1190–200. doi: 10.1089/scd.2020.0030

47. Cahuascanco B, Bahamonde J, Huaman O, Jervis M, Cortez J, Palomino J, et al. Bovine fetal mesenchymal stem cells exert antiproliferative effect against mastitis causing pathogen *Staphylococcus aureus*. *Vet Res.* (2019) 50:25. doi: 10.1186/s13576-019-09643-9

48. Carrade DD, Lane MW, Kent MS, Clark KC, Walker NJ, Borjesson DL. Comparative analysis of the immunomodulatory properties of equine adult-derived mesenchymal stem cells(). *Cell Med.* (2012) 4:1–11. doi: 10.3727/215579112x647217

49. Carrade Holt DD, Wood JA, Granick JL, Walker NJ, Clark KC, Borjesson DL. Equine mesenchymal stem cells inhibit T cell proliferation through different mechanisms depending on tissue source. *Stem Cells Dev.* (2014) 23:1258–65. doi: 10.1089/scd.2013.0537

50. Colbath AC, Dow SW, Phillips JN, McIvorath CW, Goodrich LR. Autologous and allogeneic equine mesenchymal stem cells exhibit equivalent immunomodulatory properties in vitro. *Stem Cells Dev.* (2017) 26:503–11. doi: 10.1089/scd.2016.0266

51. Caffi V, Espinosa G, Gajardo G, Morales N, Duran MC, Uberti B, et al. Pre-conditioning of equine bone marrow-derived mesenchymal stem cells increases their immunomodulatory capacity. *Front Vet Sci.* (2020) 7:381. doi: 10.3389/fvets.2020.00318

52. Espinosa G, Plaza A, Schenffeldt A, Alarcon P, Gajardo G, Uberti B, et al. Equine bone marrow-derived mesenchymal stem cells inhibit reactive oxygen species production by neutrophils. *Vet Immunol Immunopathol.* (2020) 221:109975. doi: 10.1016/j.vetimm.2019.109975

53. Hoatham WE, Thompson C, Sza-Ting L, Henson FMD. The anti-inflammatory effects of equine bone marrow stem cell-derived extracellular vesicles on autologous chondrocytes. *Vet Rec Open.* (2021) 8:e22. doi: 10.1002/vro.22

54. Harman RM, Patel RS, Fan JC, Park JE, Rosenberg BR, Van De Walle GR. Single-cell RNA sequencing of equine mesenchymal stem cells from primary donor-matched tissue sources reveals functional heterogeneity in immune modulation and cell motility. *Stem Cell Res Ther.* (2020) 11:524. doi: 10.1186/s13287-020-02043-5

55. Cortes-Araya Y, Amlion K, Rink BE, Black G, Lisowski Z, Donadeu FX, et al. Limited immune-modulating activity of porcine mesenchymal stromal cell-based therapies. *Stem Cell Res Ther.* (2016) 7:96. doi: 10.1186/s13287-016-0104-9

56. Wang L, Xu XF, Liu J, Gao K, Zeng YZ, et al. Immunogenicity and immune modulation of osteogenic differentiated mesenchymal stem cells from Bannin minipig inbred line. *Transplant Proc.* (2006) 38:2267-9. doi: 10.1016/j.transproced.2006.06.048

57. Kumar G, Hara H, Long C, Shakh H, Ayares D, Cooper DK, et al. Adipose-derived mesenchymal stem cells from genetically modified pigs: immunogenicity and immune modulatory properties. *Cytotherapy.* (2012) 14:494–504. doi: 10.3109/14653249.2011.651529

58. Khatri M, O’Brien TD, Chaththa KS, Saif LJ. Porcine lung mesenchymal stromal cells possess differentiation and immunoregulatory properties. *Stem Cell Res Ther.* (2015) 6:e22. doi: 10.1186/s13287-015-0220-0

59. Brutus-Spickenheier B, Boche J, Westenfelder C, Peimann F, Gruber AD, Alber H, et al. Critical limitations of porcine mesenchymal stem cells for regenerative medicine. *Stem Cells Dev.* (2010) 19:719–29. doi: 10.1089/scd.2009.0494

60. Yuen T, Li K, Guo L, Fan H, Zhang X. Modulation of immunological properties of allogeneic mesenchymal stem cells by collagen scaffolds in cartilage tissue engineering. *J Biomed Mater Res A.* (2011) 98:332–41. doi: 10.1002/jbm.a.33121

61. Noronha NC, Mizukami A, Caliari-Oliveira C, Cominal JG, Rocha JL, Covas DT, et al. Priming approaches to improve the efficacy of mesenchymal stem cell-based therapies. *Stem Cell Res Ther.* (2019) 10:131. doi: 10.1186/s13287-019-1224-y

62. Cote M. Oxygen in the cultivation of stem cells. *Ann N Y Acad Sci.* (2005) 1049:1–8. doi: 10.1196/annals.1334.001
Antebi B, Rodriguez LA 2nd, Walker KP 3rd, Asher AM, Kamucheka RM, Alvarado L, et al. Short-term physiological hypoxia potentiates the therapeutic function of mesenchymal stem cells. Stem Cell Res Ther. (2018) 9:265. doi: 10.1186/s13287-018-1007-x

Han Y, Ren J, Bai Y, Pei X, Han Y. Exosomes from hypoxia-treated human adipose-derived mesenchymal stem cells enhance angiogenesis through VEGF/VEGFR. Int J Biochem Cell Biol. (2019) 109:59–68. doi: 10.1016/j.biocel.2019.01.017

Jassaud J, Biais M, Calderon J, Chevaleyre J, Duchez P, Ivanovic Z, et al. Hypoxia-preconditioned mesenchymal stromal cells improve cardiac function in a swine model of chronic myocardial ischaemia. Eur J Cardiothorac Surg. (2013) 43:1050–7. doi: 10.1093/ejcts/ezs549

Liu YY, Chiang CH, Hung SC, Chian CF, Tsai CL, Chen WC, et al. Hypoxia-preconditioned mesenchymal stem cells ameliorate ischemia/reperfusion-induced lung injury. PLoS ONE. (2017) 12:e0187637. doi: 10.1371/journal.pone.0187637

Schive SW, Mirlashari MR, Hasvold G, Wang M, Jøs seesen D, Gullestad HP, et al. Human adipose-derived mesenchymal stem cells respond to short-term hypoxia by secreting factors beneficial for human islets in vitro and potentiate anti-diabetic effect in vivo. Cell Med. (2016) 9:103–16. doi: 10.3727/215517916X693401

Wang JW, Qiu YR, Fu Y, Liu J, He ZJ, Huang ZT. Transplantation with hypoxia-preconditioned mesenchymal stem cells suppresses brain injury caused by cardiac arrest-induced global cerebral ischemia in rats. J Neurosci Res. (2017) 95:2059–70. doi: 10.1002/jnr.24025

Wang W, Wang Y, Deng G, Ma J, Huang X, Yu J, et al. Transplantation of hypoxic-preconditioned bone mesenchymal stem cells retards intervertebral disc degeneration via enhancing implanted cell survival and migration in rats. Stem Cells Int. (2018) 2018:7546195. doi: 10.1155/2018/7546195

Hu Y, Chen W, Wu L, Jiang L, Qin H, Tang N. Hypoxic preconditioning improves the survival and neural effects of transplanted mesenchymal stem cells via CXCL12/CXCR4 signalling in a rat model of cerebral infarction. Cell Biochem Physiol. (2019) 37:504–15. doi: 10.1002/cbph.3423

Jiang CM, Liu J, Zhao YJ, Xiao L, An S, Gou YC, et al. Effects of hypoxia on the immunomodulatory properties of human gingiva-derived mesenchymal stem cells. J Dent Res. (2015) 94:69–77. doi: 10.1177/002203451457671

Du WJ, Reppel L, Leger I, Schenowitz C, Huiselstein C, Bensousan D, et al. Mesenchymal stem cells derived from human bone marrow and adipose tissue maintain their immunosuppressive properties after chondrogenic differentiation: role of HLA-G. Stem Cells Dev. (2016) 25:1454–69. doi: 10.1089/scd.2016.0022

Kadle RL, Abdou SA, Villarreal-Ponce AP, Soares MA, Sultan DL, David JA, et al. Microenvironmental cues enhance mesenchymal stem cell-mediated angiogenesis through VEGF/VEGF-R. J Cell Physiol. (2017) 232:2672–84. doi: 10.1002/jcp.28342

Bukowska J, Slowinska M, Cierniak P, Polak S, Wielgosz JB, et al. Hypoxia-induced mesenchymal stem/stromal cells enhance myoblast fusion during 2- and 3-dimensional culture. J Cell Mol Med. (2016) 20:20035. doi: 10.1007/s10126-020-76796-7

Archacka K, Grabowska I, Chrobota M, Gorzyńska A, Krawczyk M, et al. Hypoxia preconditioned bone marrow-derived mesenchymal stromal cells enhance myoblast fusion and skeletal muscle regeneration. Stem Cell Res Ther. (2021) 12:440. doi: 10.1186/s13287-021-00539-3

Bukowska J, Slowinska M, Cierniak P, Kowalska M, Zawilinska K, et al. Hypoxia preconditioned bone marrow-derived mesenchymal stromal cells enhance myoblast fusion during 2- and 3-dimensional culture. J Cell Mol Med. (2017) 21:1505–14. doi: 10.1111/jcmm.13820

Borges TD, Jomha NM, Mulet-Sierra A, Adesida AB. Hypoxic culture of bone marrow-derived mesenchymal stromal stem cells differentially enhances in vitro chondrogenesis within cell-seeded collagen and hyaluronic acid porous scaffolds. Stem Cell Res Ther. (2015) 6:84. doi: 10.1186/s13287-015-0075-4

Arancha K, Grabowska I, Mierzejewski B, Graffstein J, Zawilinska K, et al. Hypoxic preconditioning of human mesenchymal stem cells in combination with hyaluronic acid porous scaffolds. J Craniomaxillofac Surg. (2017) 45:414–23. doi: 10.1016/j.jcms.2016.12.011

Pfeifferinger M, Bartsch J, Hoff P, Ponomarev I, Barnewitz D, Thonn-Reinecke C, et al. Hypoxia and mesenchymal stromal cells as key drivers of initial fracture healing in an equine in vitro fracture hematology model. PLoS ONE. (2019) 14:e024276. doi: 10.1371/journal.pone.024276

Chiang ER, Ma HL, Wang JP, Liu CL, Chen TH, Hung SC. Allogeneic mesenchymal stem cells in combination with hyaluronic acid for the treatment of osteoarthritis in rabbits. PLoS ONE. (2016) 11:e0149835. doi: 10.1371/journal.pone.0149835

Chiang ER, Ma HL, Wang JP, Chang MC, Liu CL, Chen TH, et al. Use of allogeneic hypoxic mesenchymal stem cells for treatment disc degeneration in rabbits. J Orthop Res. (2019) 37:1440–50. doi: 10.1002/jor.24342

Weiss ARR, Dhahke MH. Immunomodulation by mesenchymal stem cells (MSCs): mechanisms of action of living, apoptotic, and dead MSCs. Front Immunol. (2019) 10:1191. doi: 10.3389/fimmu.2019.01191
112. Ren G, Zhang L, Zhao X, Xu G, Zhang Y, Roberts AI, et al. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. Cell Stem Cell. (2008) 2:141–50. doi: 10.1016/j.stem.2007.11.014

113. Bernardo ME, Fibbe WE. Mesenchymal stromal cells: sensors and switchers of inflammation. Cell Stem Cell. (2013) 13:392–402. doi: 10.1016/j.stem.2013.09.006

114. Noone C, Kihn A, English K, O’dea S, Mahon BP. IFN-gamma stimulated human umbilical-tissue-derived cells potentely suppress NK activation and resist NK-mediated cytotoxicity in vitro. Stem Cells Dev. (2013) 22:3003–14. doi: 10.1089/scd.2013.0028

115. Bulati M, Miceli V, Gallo A, Amico G, Caricione C, Pampalone M, et al. The immunomodulatory properties of the human amnion-derived mesenchymal stromal/stem cells are induced by INF-gamma produced by activated lymphomonocytes and are mediated by cell-to-cell contact and soluble factors. Front Immunol. (2020) 11:54. doi: 10.3389/fimmu.2020.00534

116. Park SG, An JH, Li Q, Chae HK, Park SM, Lee JH, et al. Feline adipose tissue-derived mesenchymal stem cells pretreated with IFN-gamma enhance immunomodulatory effects through the PGE2 pathway. J Vet Sci. (2021) 22:e16. doi: 10.4142/jvs.2021.22.e16

117. Yu Y, Yoo SM, Park HH, Baek SY, Kim YJ, Lee S, et al. Preconditioning with interleukin-1 beta and interferon-gamma enhances the efficacy of human umbilical cord blood-derived mesenchymal stem cells-based therapy via enhancing prostaglandin E2 secretion and indoleamine 2,3-dioxygenase activity in dextran sulfate sodium-induced colitis. J Tissue Eng Regen Med. (2019) 13:1792–804. doi: 10.1002/term.2930

118. Yang R, Huang H, Cui S, Zhou Y, Zhang T, Zhou Y. IFN-gamma promoted exosomes from mesenchymal stem cells to attenuate colitis via miR-125a and miR-125b. Cell Death Dis. (2020) 11:603. doi: 10.1038/s41419-020-02788-0

119. Luk F, Carreras-Planella L, Korevaar SS, De Witte SFH, Betjes MGH, et al. Inflammatory conditions dictate the effect of mesenchymal stem or stromal cells on B cell function. Front Immunol. (2017) 8:1042. doi: 10.3389/fimmu.2017.01042

120. Duijvestein M, Woldenberg ME, Welling MM, Hennink S, Molendi jk I, Abeliuk, M., Vega-Letter, A. M., et al. (2016). Toll-like receptor 3 pre-conditioning increases the therapeutic efficacy of umbilical cord mesenchymal stromal cells in a dextran sulfate sodium-induced colitis model. Cytotherapy 18, 630–641. doi: 10.1016/j.jcyt.2016.02.002

121. Qiu Y, Guo J, Mao R, Chao K, Chen BL, He Y, et al. TLRI3 preconditioning enhances the therapeutic efficacy of umbilical cord mesenchymal stem cells in TNBS-induced colitis via the TLRI3-Jagged-1-Notch-1 pathway. Mucosal Immunol. (2017) 10:727–42. doi: 10.1038/mi.2016.78

122. Cassano JM, Schnabel LV, Goodeal MB, Fortier LIA. The immunomodulatory function of equine MSCs is enhanced by priming through an inflammatory microenvironment or TLRI3 ligand. Vet Immunol Immunopathol. (2018) 195:33–9. doi: 10.1016/j.vetimm.2017.10.003

123. Chow L, Johnson V, Impastato R, Coy J, Strumpf A, Dow S. Antibacterial activity of human mesenchymal stem cells mediated directly by constitutively secreted factors and indirectly by activation of innate immune effector cells. Stem Cells Transl Med. (2020) 9:235–49. doi: 10.1002/scit.19-0092

124. Pezzanite LM, Chow L, Johnson V, Griffenhagen GM, Goodrich L, Dow S. Toll-like receptor activation of equine mesenchymal stem cells to enhance antibacterial activity and immunomodulatory cytokine secretion. Vet Surg. (2021) 50:858–71. doi: 10.1111/vsu.13628

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