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Does mesenchymal stem cell's secretome affect spinal sensory circuits? Implication for pain therapies

Francesco Ferrini, Esri H. Juárez, Adalberto Merighi*

Mesenchymal stem cells (MSCs) are multipotent adult stem cells of mesodermal origin that can be isolated from various tissues, including bone marrow, tooth pulp, adipose tissue, and umbilical cord. MSCs have gained significant attention in regenerative medicine due to their ability to modulate the immune system and favor tissue repair. MSCs enrich the medium in which they are cultivated with a broad range of bioactive molecules, including growth factors, cytokines, chemokines, enzymes, nucleic acids, and extracellular vesicles that collectively compose the MSC secretome. An increasing number of pre-clinical studies suggest that delivering *in vivo* an MSC-conditioned medium (i.e., the medium collected from MSC cultures after at least 3 days of exposure) exerts neuroprotective and anti-inflammatory effects in a variety of neurological conditions, including chronic pain. Importantly, since therapies based on MSC transplantation are highly impaired by the limited cell survival in the host and by the potential occurrence of immunological adverse responses, MSC secretome represents a safer and more viable alternative to these therapies.

Therefore, we here revise the state of the art of the effects of MSC secretome in chronic pain, analyze its involvement in the modulation of spinal nociceptive circuits and neuroinflammatory processes, and discuss the potential therapeutic opportunities that could be linked to its use in clinical settings.

MSC secretome in chronic pain models: The antinociceptive effect of an MSC-conditioned medium has been described in different preclinical studies in rodents and a few clinical trials in humans. In particular, local delivery of MSC secretome has been largely demonstrated to reduce pain hypersensitivity in different models of inflammatory pain, such as osteoarthritis. In a limited number of studies, the anti-hyperalgesic effect has been also demonstrated by systemic administration.

Specifically, the secretome of human adipose-derived MSCs was found to relieve osteoarthritic pain when delivered both locally and systemically, with systemic administration being the most effective (Amodeo et al., 2021). Moreover, in model cystitis, pain relief was obtained by intrathecal delivery of extracellular vesicles isolated by centrifugation from the conditioned medium of human umbilical cord MSCs (Zhang et al., 2022). Taken together, these observations indicate that the site of action of the MSC secretome to reduce inflammatory pain might not be restricted to the peripheral tissues only, but might also involve nociceptive pathways in the central nervous system.

The relevance of central mechanisms in the antinociceptive effect of MSC secretome has been consistently reported in neuropathic pain. In animal models of neuropathic pain induced either by sciatic nerve ligation or diabetes, the intravenous administration of conditioned medium from bone marrow or adipose tissue MSCs displayed anti-allodynic properties similar to that obtained by MSC transplantation (Brini et al., 2017; Evangelista et al., 2018; Gama et al., 2018). Likewise, delivering intraperitoneally the MSC-conditioned medium was shown to restore normal nociceptive behavior in rats with chronic constriction injury (Masoodifar et al., 2021). Interestingly, the intrathecal delivery of extracellular vesicles purified from MSCs was also found to attenuate neuropathic pain symptoms (Shiue et al., 2019; Gao et al., 2023a, b), thus highlighting the relevance of spinal cord circuits as a target for MSC secretome.

MSC secretome and spinal neuroinflammation: The spinal dorsal horn is a key site for the processing of sensory and nociceptive signals directed to the brain, and neuroinflammation in this region is often associated with chronic pain conditions. The secretome of MSCs has immunomodulatory properties and may attenuate neuroinflammation by inhibiting the activation of glial cells and the subsequent release of inflammatory mediators. Indeed, MSC-conditioned medium was found to reduce the level of classical pro-inflammatory cytokines (such as interleukin-1β, tumor necrosis factor-α, and interleukin-6) in the spinal cord of neuropathic animals, while anti-inflammatory cytokines (such as interleukin-10, and transforming growth factor-β) were enhanced (Brini et al., 2017; Evangelista et al., 2018; Gama et al., 2018). Activated microglia, the resident macrophages in the central nervous system, represent the major source of inflammatory cytokines in neuroinflammatory processes. MSC secretome reduces spinal microglia activation in animals with neuropathic pain and restores resting phenotypes (Gao et al., 2023a, b). The switch of spinal microglia to a pro-inflammatory phenotype in neuropathic pain has been consistently associated with an increased expression of ionotropic purinergic receptors, such as P2X4 and P2X7 (Masoodifar et al., 2021). Interestingly, the increase of both receptors is inhibited by the MSC secretome (Masoodifar et al., 2021). The intracellular pathways underlying the inhibition of microglia activation have been finely dissected by Gao and collaborators by delivering MSC exosomes (Gao et al., 2023a, b). The authors have found that MSC-derived extracellular vesicles promote autophagy in microglia, an intracellular degradation process that inhibits microglia activation. Specifically, extracellular vesicles interfere with the main signaling pathway underlying microglia activation (PI3K/AKT/mTOR pathway) which in turn favors the autophagic processes. In parallel, extracellular vesicles also inhibit the microglial pathways initiated by the toll-like receptor 2, which promotes the synthesis and release of pro-inflammatory cytokines.

While the effects of MSC secretome on microglia and their impact on chronic pain are well defined, the involvement of other glial cells, and in particular astrocytes, has been poorly investigated. On one side, an increased expression of both microglia and astrocytic markers (Iba1/CD68 or GFAP, respectively) has been described in both inflammatory and neuropathic pain models, and this upregulation was reduced following the delivery of conditioned medium from human umbilical cord MSCs (Shiue et al., 2019; Zhang et al., 2022). On the other side, we found that applying the conditioned medium from canine adipose tissue MSCs on spinal cord organotypic cultures from healthy mice induced upregulation of astrocytic markers (Wood et al., 2021). Of course, differences in the source of MSCs may explain different experimental outcomes, thus more studies are needed to understand the impact of MSC secretome not only in pathological conditions but also on healthy tissues.

MSC secretome and spinal neurons: Spinal dorsal horn neurons are critically involved in the transmission of nociceptive information to the brain. Surprisingly, the impact of MSC secretome on neuronal activity has been largely overlooked. Although some pieces of evidence indicate that MSC secretome attenuates the increased expression of markers of neuronal activation or neuronal inflammasome following chronic pain (Shiue et al., 2019; Zhang et al., 2022), the direct effect on firing or synaptic activity has received very little attention. We have recently addressed this point by analyzing the electrophysiological and functional properties of dorsal horn neurons in organotypic cultures of the mouse spinal cord exposed to the secretome from bone marrow-derived ST2 MSCs (Juarez et al., 2023). The secretome from these cells induced an overall

increase of excitability in the spinal dorsal horn by acting both on active membrane properties and glutamatergic synaptic input of a heterogenous population of neurons, likely including both excitatory and inhibitory interneurons. Moreover, we observed a shift of calcium waveforms in the neuronal network from single spikes to multi-peaked bursts. An increased synchronized activity supports the role of the secretome in promoting synaptic connectivity, which is typically observed in network maturation. Considering that spinal organotypic slices are a deafferented system, whose circuits undergo some re-wiring *in vitro*, our results support a neurotrophic function of the MSC secretome to facilitate proper connectivity restoration between neurons. Altogether, these effects might be relevant to restoring the integrity of spinal circuits when altered by pathological conditions such as nerve or spinal cord injury and maintaining an optimal imbalance between inhibition and excitation. A putative mechanism taking into account both trophic and anti-inflammatory effects of MSC secretome on injured spinal dorsal horn is illustrated in **Figure 1**.

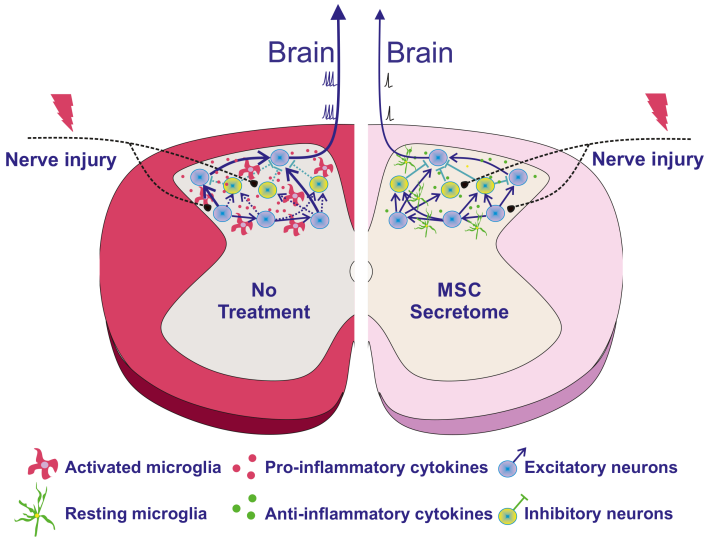

Figure 1 | **Proposed mechanism by which MSC secretome restores dorsal horn circuits in models of nerve injury and reduces neuroinflammation.**
After nerve injury spinal circuits undergo deep alterations: activated microglia release pro-inflammatory cytokines and trophic factors that are known to disrupt local circuits causing disinhibition and favoring nociceptive transmission to the brain. As discussed in this perspective article, MSC secretome recovers the resting microglial phenotype in nerve-injured animals, thus reducing neuroinflammation and the associated increase in pain hypersensitivity. Based on our findings in organotypic spinal cord slices, we expect that the trophic properties of the secreotme by promoting connectivity of local spinal circuits, may in turn restore a correct balance between inhibition and excitation. Created with CorelDRAW X6.
MSC: Mesenchymal stem cell.

Therapeutic opportunities for chronic pain: Despite promising preclinical evidence, the investigations of treatments for chronic pain based on MSC secretome are still in their early stages, and more research is needed to fully understand the underlying mechanisms and to optimize the therapeutic potential.

MSCs release in the medium a variety of molecules and a major challenge in the field is to discriminate what is relevant in the secretome composition for pain management and what is unimportant or even deleterious. A summary of the main molecular effectors identified in different types of MSC secretomes and associated with chronic pain models is reported in **Table 1**.

The analysis of the molecular content of the conditioned media in different studies highlighted some differences in the composition, probably due to differences in the method used for screening or the use of different sources of MSCs. However, chemokines, cytokines, and growth factors are consistently reported (Cantinieaux et al., 2013; Gama et al., 2018; Shiue et al., 2019; Amodeo et al., 2021). In particular, the proteomic analysis of conditioned medium by Amodeo and collaborators revealed a high content of immunomodulatory factors such as ADAM10, annexin, clusterin, gelsolin, and pentraxin 3 that are known to reduce inflammatory processes (Amodeo et al., 2021). Moreover, Gama and collaborators identified the hepatocyte growth factor and the vascular endothelial growth factor (VEGF) in the secretome of bone marrow-derived MSCs, which are known to have both neuroprotective and antinociceptive effects (Gama et al., 2018). Cantinieaux and collaborators confirmed the presence of VEGF, but in addition, they reported also the brain-derived neurotrophic factor and the nerve growth factor, which instead may exert pro-nociceptive effects. The neurotrophic factor content in conditioned media is of potential interest for therapeutic intervention, but its application is highly hampered by the short half-life of these molecules when delivered systemically and by the limited crossing of the blood-brain barrier which reduces the efficacy in the central nervous system. Appropriate ways of storage (e.g. lyophilization), routes of administration (e.g. intranasal delivery), and vehicles (e.g. controlled delivery) need to be envisaged to improve the efficacy of MSC secretome and reduce possible unwanted effects. Besides, the variability in conditioned medium composition obtained from different laboratories/cell lines/animals, or the lack of information on the medium content, are other caveats that need some refinement and standardization for more rational use of MSC secretome in clinical settings.

Another interesting avenue is represented by the isolation of extracellular vesicles from the conditioned medium. Extracellular vesicles are thought to be responsible for most of the paracrine functions associated with the MSC secretome, as they can carry different types of molecules, including proteins, metabolites, nucleic acids, and trophic factors (Shiue et al., 2019). Importantly, it has been shown that extracellular vesicles also contain miRNA which exerts antinociceptive effects in neuropathic pain by their immunomodulatory function on microglia (Gao et al., 2023a). Extracellular vesicles are very stable, exhibit very low immunogenicity and good biocompatibility, and can be easily engineered to carry specific nucleic acids or drugs to the nervous system.

Altogether, the MSC secretome represents an attractive cell-free option for chronic pain treatment. Yet, most of the antinociceptive effects so far described lack a strong understanding of the underlying mechanisms. More efforts are needed to elucidate the molecular content of MSC secretomes from different sources and clarify their impact on neuronal transmission under healthy and pathological conditions.

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Table 1 | **Summary of key spinal mechanisms associated with MSC secretomes in chronic pain models and the identified effectors**

Animal model of pain	Animal species	MSC origin	FS or purified EV	Identified factor	Spinal mechanism	Reference
Osteoarthritic pain	C57BL/6J mouse	Human adipose tissue	FS	ADAM10 annexin clusterin gelsolin pentraxin	↓ GFAP ↓ CD68 ↓ Pro-inflammatory cytokines	Amodeo et al., 2021
Interstitial cystitis	Sprague-Dawley rat	Human umbilical cord	EV	–	↓ NLRP3 inflammasome ↓ TLR4/NF-κB pathway ↓ GFAP ↓ IBA1 ↓ Pro-inflammatory cytokines	Zhang et al., 2022
Diabetic neuropathy	C57BL/6J mouse	Human adipose tissue	FS	–	↓ Pro-inflammatory cytokines ↑ Anti-inflammatory cytokines	Brini et al., 2017
Diabetic neuropathy	C57Bl/6 mouse	Mouse bone marrow	FS	–	↓ Pro-inflammatory cytokines ↑ Anti-inflammatory cytokines ↓ GFAP ↓ IBA1	Evangelista et al., 2018
Spinal cord injury	Wistar rat	Rat bone marrow	FS	VEGF, BDNF, NGF	↑ Angiogenesis ↓ Apoptosis - GFAP - CD11b	Cantinieaux et al., 2013
Partial sciatic nerve ligation	C57Bl/6 mouse	Mouse bone marrow	FS	HGF, VEGF chemerin angiopoetin-1	↓ Pro-inflammatory cytokines ↑ Anti-inflammatory cytokines	Gama et al., 2018
L5/6 spinal nerve ligation	Sprague–Dawley rat	Human umbilical cord	EV	VEGF, FGF-2 angiopoietin-2	↓ Fos ↓ GFAP ↓ IBA1 ↓ Pro-inflammatory cytokines ↑ Anti-inflammatory cytokines ↑ BDNF ↑ GDNF	Shiue et al., 2019
Chronic constriction injury of the sciatic nerve	Wistar rat	Rat bone marrow	FS	–	↓ P2X4 ↓ P2X7	Masoodifar et al., 2021
Chronic constriction injury of the sciatic nerve	Sprague–Dawley rat	Human umbilical cord	EV	miR-99b-3p	↓ TLR2/MyD88/NF-κB ↓ PI3K/AKT/mTOR ↓ Rsad2 ↓ CD68 ↓ Pro-inflammatory cytokines ↑ Autophagy	Gao et al., 2023a,b

ADAM10: A disintegrin and metalloproteinase domain-containing protein 10; AKT: protein kinase B; BDNF: brain-derived neurotrophic factor; CD11b: cluster of differentiation 11b; CD68: cluster of differentiation 68; EV: extracellular vesicle; FGF-2: fibroblast growth factor-2; Fos: Fos proto-oncogene; FS: full secretome; GFAP: glial fibrillary acidic protein; HGF: hepatocyte growth factor; IBA1: ionized calcium-binding adapter molecule 1; miR-99b-3p: microRNA 99b-3p; MSC: mesenchymal stem cell; mTOR: mechanistic target of rapamycin; MyD88: myeloid differentiation primary response 88; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; NGF: nerve growth factor; NLRP3: NLR family pyrin domain containing 3; P2X4: P2X purinoceptor 4; P2X7: P2X purinoceptor 7; PI3K: phosphoinositide 3-kinase; Rsad2: radical S-adenosyl methionine domain containing 2; TLR2: toll-like receptor 2; TLR4: toll-like receptor 4; VEGF: vascular endothelial growth factor.

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