Gallant

How Mesenchymal Stem Cells Affect Longevity

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Introduction

Biological aging is defined as physiological decline over time.[1] This process occurs at a molecular level, involving genetic and cellular derangements such as telomere attrition, epigenetic dysregulation, and accumulation of toxic protein aggregates and reactive oxygen species. As genes and cells are effectively the blueprints and building blocks of life, their abnormal form and function negatively impact the health of tissues, organs, and organ systems, until, ultimately, the quantity and severity of such abnormalities become incompatible with normal physiology, resulting in death.

The converse of this process is regeneration, which involves the reversal of molecular aberrations and the replacement of lost functional units.[1] This occurs to some degree in all animals; most evidently through healing of physical injuries. Over a lifetime, however, aging, like entropy, is a naturally dominant process. Because of this, regenerative therapy has been investigated as a means of tilting the scales against aging by sustaining health and extending longevity.

Stem cells are an integral part of regeneration, both naturally, (i.e. through healing), and potentially for regenerative therapy, as stem cells serve as progenitors for all types of cells in the body, making them potential replacements for cells damaged or lost through aging.[2]

Mesenchymal stem cells (MSCs) are clonal, plastic adherent, non-hematopoietic cells that give rise to mesodermal cell lineages.[3] *In vitro*, and possibly *in vivo,* they have also been shown to differentiate into endodermal and neuroectodermal cells.[4] First harvested from bone marrow, MSCs have since been isolated from a wide range of tissue types, such as adipose and reproductive tissue.[5,6] MSCs are an attractive tool for regenerative therapy because they are easily harvested, rapidly expanded *in vitro*, and their broad differentiation potential suggests equally broad clinical applications.[7]

In the United States, veterinarians take an oath to relieve animal suffering, which can require prioritization of quality of life over quantity of life.[8] When quality of life becomes too poor, animals may be euthanized, instead of delivering treatments aimed at extending lifespan as long as possible. For this reason, quality and quantity of life are intrinsically linked in veterinary medicine, since quality of life often dictates quantity of life, with poorer quality of life predicting shorter lifespan.

This paradigm leads to many instances of euthanasia due to non-life-threatening diseases. For example, in a 2017 study investigating companion animal deaths at a clinic in New Zealand, 91% of dogs that died

over a two-year period were euthanized.[9] Of these, almost one-third (32%) were euthanized as a result of decreased quality of life due to osteoarthritis. Similarly, diseases such inflammatory bowel disease and keratoconjunctivitis sicca (KCS or dry eye), which are non-life-threatening in humans, can be in companion animals. Therefore, even if MSCs can't stop the systemic, molecular processes of aging, such as telomere attrition, their efficacy in treating specific chronic diseases may consequently allow them to extend longevity.

The present article will review literature concerning the relationship between MSCs and longevity. When possible, the focus will be on articles addressing this as a direct relationship, although major research concerning chronic diseases will also be considered. The next section offers a brief mechanistic overview of aging and the role of stem cells in this process, followed by a review of experimental studies evaluating the effect of MSCs on lifespan.

Mechanisms of aging

General information

Aging is an extremely complicated process that occurs at multiple levels, and a variety of mechanisms have been proposed to explain how it happens, with just one of these processes being stem cell exhaustion. For a comprehensive review of such mechanisms, consider reading "The hallmarks of aging," by López-Otín and colleagues, which more closely explores the following nine mechanisms of aging:[10]

1) Stem cell exhaustion – stem cell function diminishes over time, limiting capacity for renewal

2) Genomic instability – genetic damage accumulates over time due to naturally occurring endogenous processes, such as accumulation of reactive oxygen species, and exogenous processes, such as exposure to chemical agents

3) Telomere attrition – telomeres are the protective endcaps of DNA strands that shorten over time, thereby limiting the replicative ability of DNA

4) Epigenetic alterations – like DNA itself, processes involved in DNA expression become abnormal over time

5) Loss of proteostasis – also called "protein homeostasis," proteostasis refers to stabilization of protein structure; as this becomes less effective over time, abnormal protein formations may occur, as seen with conditions such as Alzheimer's and Parkinson's disease

6) Deregulated nutrient sensing – over time, cells are less able to sense and respond to fuel sources, like glucose

7) Mitochondrial dysfunction – the cell's power generator degrades over time, leading to reduced ATP output and increased electron leakage

8) Cellular senescence – the cell cycle stops in older cells, which is due to telomere shortening and other processes, such as DNA damage

9) Altered intercellular communication – neuronal, endocrine, and neuroendocrine signaling becomes disrupted over time, resulting in systemic inflammation and the decline of the adaptive immune system

Stem cell exhaustion and microenvironmental dysregulation

Over time, stem cells undergo functional decline in which their regenerative capacity decreases.[11] Specifically, this decline involves greater susceptibility to oxidative stress and the accumulation of toxic aggregates, which eventually result in apoptosis, necrosis, or autophagy.[2] Several drivers contribute to this process, including microenvironmental influences, DNA damage, mitochondrial dysfunction, epigenetic alterations, and telomere attrition.[2,12]

The stem cell microenvironment, or niche, has been a focus of particular interest in the area of aging. In addition to intrinsic self-regulation, stem cells receive signals from regulatory cells within their niche.[13,14] As niche cells age, their numbers decrease, while proinflammatory cytokines, such as IL-6 and TNF-α, become more common.[2] Furthermore, changes occur in concentrations of circulating factors such as insulin, IGF-1, and TGF-β, causing niche cells to malfunction and send altered signals to stem cells.[12] While MSC transplantation focuses on replacing aged MSCs, future therapy may focus on manipulating the microenvironment to regenerate existing stem cells and prolong life.[13,15]

The effect of MSC transplantation on lifespan

The potential for MSC transplants to indirectly extend lifespan by slowing disease progression has been demonstrated in a variety of human diseases, such as amyotrophic lateral sclerosis (ALS), T-cell lymphoma, and Crohn's disease.[16-18]

In a controlled trial involving a mouse model of ALS, which is characterized by progressive neurodegeneration, intramuscular injection of allogeneic bone marrow-derived MSCs (BM-MSCs) slowed disease progression and extended lifespan, likely due to the release of neurotrophic factors from stem cells that helped sustain neuromuscular junctions.[18]

A retrospective human study involving patients with peripheral T-cell lymphoma also reported life-extending results.[17] Those who received intravenous autologous stem cell transplantation (ASCT; source not described) after first-line chemotherapy had a significantly better rate of 5-year progression free survival (65% vs 44%; *P* = .012) and overall survival (74% vs 65%; *P* =.046) than patients who did not receive ASCT.

Allogeneic, adipose-derived MSCs (AD-MSCs) have also led to better disease outcomes in humans. One such trial was a phase 3, randomized, double-blind controlled study by Panés and colleagues involving 212 patients with perianal fistulas secondary to Crohn’s disease.[16] Patients in the treatment group were injected with 120 million AD-MSCs into the tissues surrounding fistula tracts and internal openings, resulting in a rate of remission significantly higher than in the placebo group (50% vs 34%; *P* = .024). Perianal fistulas are generally not life-threatening in humans, so survival differences were not reported; however, in the veterinary world, canine perianal fistulas can be notoriously difficult to treat and exceedingly painful, making them a potential chronic disease target that could save afflicted dogs from euthanasia.[19]

In fact, this potential was demonstrated by an uncontrolled, prospective trial involving 6 dogs with perianal fistulas.[20] Dogs were treated in a similar way to the above trial, with injections into tissues surrounding fistulous tracts. Human embryonic stem cell (hESC)-derived MSCs were used, at a dose of 20 million cells per dog. After 3 months, all 6 dogs had resolution of fistulas; however, at 6 months, 2 of

the dogs had recurrence, albeit to a lesser degree than initial presentation. Since perianal fistulas almost never spontaneously resolve, the investigators described these results as “striking,” even without a control group. They also suggested that multiple injections may be necessary to completely resolve lesions, although this was not pursued because of possible sensitization due to the xenogeneic MSC source. As it was, the protocol appeared safe, with no serious adverse events. One dog had mild, self-limiting perianal erythema, although the investigators suggested that this could have been due to shaving and site prep.

An array of other chronic conditions in animals have been shown to improve with MSC transplants, including immune-mediated disease, neurologic injury, and degenerative conditions.[4,21-29]

In high-fat diet-induced diabetes in mice, two intravenous injections of allogeneic, human-derived AD-MSCs were given at a dose of 42 million cells per kilogram.[21] This resulted in improved glucose tolerance and blood glucose homeostasis, and reduced body weight. The investigators cited two mechanisms of action: direct differentiation of MSCs into pancreatic islet cells capable of producing insulin, and downregulation of IL-1 and TNF-α in the pancreas, which exemplify renewal of cells and immunomodulation, respectively.

In dogs, at least 3 studies have shown that MSCs can improve motor function after spinal cord injury, a risk factor for euthanasia.

The first of these studies, from 2009, was a prospective controlled trial involving 11 laboratory dogs that underwent anesthetized induction of spinal cord injury via epidural balloon catheter.[4] Dogs were randomized into three groups: control (no treatment), vehicle (phosphate buffered saline [PBS]), and MSC (treatment with allogenic AD-MSCs and saline). One week after spinal cord injury, dogs in the MSC group were given 1 million AD-MSCs suspended in PBS, injected directly into the injury site, followed by 8 weeks of gait and somatosensory evoked potential assessments until euthanasia at week 9. Two weeks after treatment, dogs in the MSC group had significantly better gait and nerve conduction scores than dogs in the two control groups, while the two control groups showed no differences in outcomes between one another. Luxol fast blue staining and immunohistochemistry suggested that neurological improvements were at least partially due to neuronal differentiation.

In 2014, another neurologic study was conducted, this time involving dogs with naturally occurring chronic spinal cord injury and no control animals.[22] Seven dogs were enrolled, all with spinal cord lesions secondary to intervertebral disk disease (IVDD) or trauma. Each was injected with 1 million allogeneic MSCs (derived from fetal bone marrow) into the spinal medulla, both intra-lesionally and peri-lesionally. Three months after the procedure, all dogs showed improved sensory and locomotor function, including increased movement of hind limbs, greater tail tone, ability to stand upright, and ability to take small steps. Five out of 7 dogs also regained pain reflexes and fecal continence.

A third neurologic study, also conducted in 2014, involved 4 dogs with IVDD that had no neurologic improvements 6 months after initial decompressive surgery (hemilaminectomy).[23] Dogs were injected with 1 million autologous BM-MSCs per cubic centimeter of spinal cord lesion, then followed for 18 months. Two out of 4 dogs recovered conscious reflexes and had improved intestinal and urinary bladder function, while 3 out of 4 dogs showed clinically relevant improvements in motor function. These clinical gains were not associated with changes on MRI.

More recently, research has focused on whether MSC transplantation prolongs life in aged but otherwise healthy animals.

MSCs slow aging by enhancing cell and tissue regeneration and by improving organ function, although exact mechanisms of these processes remain unclear.[30] Studies have shown that MSCs and related progenitor cells can improve cardiac function;[31] postpone reproductive failure;[32] delay lung, kidney, and colon pathology;[33] improve offspring survival;[32] and extend lifespan.[30]

In a study from 2010 by Li and colleagues, allogeneic, fetal-derived BM-MSCs were intravenously injected into 15-month-old female mice.[33] Compared with control mice, these treated mice had increased median life span and delayed pathology of the skin, heart, lung, kidneys, and colon. Further details are unavailable because the article, other than the abstract, is in Chinese.

An American study from 2009 also found promising results using BM-MSCs in mice.[32] Following treatment with cytotoxic drugs, mice in the treatment group were intravenously injected with 15-30 million allogenic BM-MSCs on a monthly basis. Compared with mice injected only with vehicle (PBS), treated mice were more likely to maintain fertility at an older age (52% vs 31%) and give birth to viable offspring (71% vs 33%).

Improved cardiac angiogenesis was demonstrated in 2002 by Edelberg and colleagues.[31] First, old mice were intravenously injected with allogeneic, endothelial precursor cells (which may be differentiated from MSCs), either from young or old donors. Then, both groups underwent cardiac allograft transplantation to determine angiogenic potential, which is typically lost in older mice. Results showed that 15 out of 16 older mice treated with 1 million precursor cells from young mice maintained their cardiac allograft transplants, compared with none of the 6 mice treated with the same amount of cells from older mice. These results suggest that stem cells and related progenitor cells harvested from older animals may not maintain the same therapeutic potential as cells from younger animals.

This concept of using younger stem cells in older animals is further supported by a 2011 study, in which researchers aimed to evaluate the effect of allogeneic BM-MSCs on the progression of osteoporosis in old female mice.[30] The mice were irradiated, then transplanted with 1 million MSCs from either young or old donor mice. In addition to slowing the progression of osteoporosis, the transplants from young donors prolonged lifespan 125 days longer than transplants from old donors (890 vs 765 days; P = .009).[30] Of note, the average lifespan of mice that received BM-MSCs from old donors and the lifespan of the control group were the same (P = .846).[30]

Young rats showed similar improvements when two types of MSC transplants were compared.[34] Human amniotic membrane-derived mesenchymal stem cell transplants were compared with adipose tissue-derived mesenchymal stem cell transplants.[34] Both extended lifespan (23.4% for amniotic membrane-derived mesenchymal stem cells vs 31.3% for adipose tissue-derived mesenchymal stem cells).[34] Additionally, cognitive and physical functions improved in both groups.[34]

In 2019, Kovina and colleagues found that MSC transplantation prolonged life even without the myeloablative conditioning traditionally used in bone marrow transplants.[35,36] Following bone marrow transplants from syngeneic young mice, the lifespan of non-myeloablative older mice increased by about 31% and their survival time from the beginning of the transplantation increased by approximately 3-fold.[35] Importantly, the increased lifespan was accompanied by a subjectively observed good quality of

life.[35] These results support findings from a 2013 study by the same researchers where mean survival time increased by 39% in mice after MSC transplantation.[36]

Despite the promising results of the 2019 study, some design limitations suggest that a conservative interpretation is needed. These limitations include a disparity of group sizes (the control group contained 20 mice, whereas the experimental group contained 51), a lack of control injections, a lack of a myeloablative control group, and the classification of injection-related embolic deaths as natural deaths. Most importantly, the results are reported differently in different sections of the paper; mouse lifespan is variously reported as having increased by 28 ± 5%, 31 ± 5% and 30%, and the survival time as having increased by both 2.8 ± 0.3-fold and 3.25 ± 0.3-fold.[35] Additionally, minor typographical and referencing errors appear and no *P* values are provided. Taken together, these issues suggest that the article may not have undergone a rigorous and ethical peer-review process.

Still, multiple animal studies demonstrate the lifespan-increasing effect of MSCs, as previously discussed.[30,33,34,37] Unfortunately, direct extension of lifespan has not been studied in dogs.

In humans, allogeneic MSC transplants improve markers of frailty syndrome, which is characterized by age-related decreases in physical endurance and strength, along with an increased concentration of inflammatory biomarkers and shortened lifespan.[38,39] This benefit was demonstrated in 2017 by Tompkins and colleagues through a randomized, double-blind, placebo-controlled phase 2 study.[38] Thirty patients were randomized to receive placebo or intravenous allogeneic BM-MSC transplants of 100 or 200 million cells. No treatment-associated serious adverse events were seen in either group after 30 days. Immunologic markers of frailty improved in both groups and physical performance improved in the 100 million group. The reason for the dosing response was unclear, but researchers aim to clarify the results in a future study.

While animal and human studies describe the lifespan-increasing properties of MSC transplantation, MSC lysate administration may do the opposite.[40] Middle-aged rats injected with adipose-derived MSC lysate (a solution of cell contents instead of entire transplanted cells) had a shorter average lifespan, decreased activity, and greater bone loss compared with those injected with saline.

However, studies have also shown that both MSC and MSC lysate transplants improve glycemic control in mice fed a high-fat diet.[41,42] Researchers postulate that MSC lysate favors cell regeneration without promoting cell clearance, creating a disparity between the two processes, thereby shortening lifespan.

MSCs and cancer

The role of MSCs in tumor pathogenesis is controversial, although some evidence suggests that MSCs contribute to tumor progression.[7] Tumors induce an inflammatory microenvironment similar to injury and MSCs are recruited to tumor sites with great affinity. From there, they exert both direct and indirect actions on cancer cells, with immunomodulatory effects on tumor progression. Studies suggest MSCs promote various stages of tumor pathogenesis, contributing to proliferation, invasion, metastasis, and angiogenesis.[7,43,44] As a result, MSC transplants may shorten lifespan due to their stimulatory effects on oncogenesis.

However, the current body of evidence is conflicting. Although the majority of studies show that MSCs stimulate oncogenesis, some show that MSCs inhibit tumor progression.[45-51] This discrepancy may relate

to a disparity in experimental conditions (treatment endpoints, tissue origins, donor variability, injection sites, and transplant timing).[7,44] Given the conflicting results, it is unclear whether MSCs enhance or inhibit tumor pathogenesis, or possibly both.[44] More research is needed to uncover how MSCs affect cancer and whether transplants can shorten lifespan.

Summary

MSC transplantation is a promising avenue for regenerative medicine. Studies show encouraging results in disease conditions in both animals and humans. This is particularly important for companion animals, as the chronic conditions that respond to MSC transplants may be life-limiting due to diminished quality of life. In healthy animals, rodent studies show a lifespan-enhancing effect with MSC transplantation. Despite the present uncertainty regarding the role MSCs play in oncogenesis, MSC therapy is considered a safe and exciting area of therapeutic research, promising to enhance both health and lifespan.

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