■Short Review Article

Safety and efficacy of human adipose-derived mesenchymal stem cell conditioned media

Mohammad Ghazizadeh

Abstract

In recent years, mesenchymal stem cell therapy has attracted much attention due to their capability of homing into the site of injury and differentiating into multiple tissue types. However, it has been revealed that stem cells do not survive for a long time after administration, and that the advantages of mesenchymal stem cell therapy could be due to their secretome namely biologically active substances that they produce, which play a significant role in the regulation of key cellular processes. Therefore, conditioned media from the mesenchymal stem cell cultures which contain such materials may present a highly effective and desirable option for use in regenerative medicine therapies. Many studies in animal models and a few in humans have shown safety and efficacy of its application. So far, no serious adverse effects has been reported. The advantages of conditioned media include easy manufacturing, storage, handling, and longer shelf life. In addition, it provides a ready-to-use biological material. However, in order to establish the safety and efficacy of such material, strict regulatory measures for manufacturing and quality control, and standardization protocol will be mandatory.

Key words　Adipose-derived mesenchymal cells, conditioned medium, safety, efficacy

(WAARM Journal, 2018; 1: 21–24)

Short review

The process of stem cell isolation and proliferation begins stepwise with 1) obtaining tissue or cells from the patient or donor, for example fat tissue in case of adipose-derived mesenchymal stem cells; 2) preparation of stromal vascular fraction; 3) culturing in animal serum-free media, and 4) isolation and collection of stem cells for use. In this process, during cell growth and proliferation in culture, stem cells release various substances in the media which is termed secretome of the stem cells. This media is called conditioned media and is collected and preserved for use.

Therefore, conditioned media contain secretome of stem cells and its composition include soluble proteins, free nucleic acids, lipids and extracellular vesicles such as apoptotic bodies, microparticles and exosomes. The secretome of individual cells and tissues is specific, and alters in response to fluctuations in physiological states or pathological conditions. More specifically, conditioned media contains various biologically active substances and structures, such as cytokines, growth factors, enzymes, microvesicles, exosomes and genetic materials.

So far, in many experimental animal studies effective therapeutic value of stem cell conditioned media has been demonstrated (Table 1). For example, conditioned media from various sources of mesenchymal stem cells such as bone marrow, umbilical cord, dental pulp, adipose tissue and so on have been widely studied in animal models of a variety of disease conditions. According to the reported studies, no serious adverse effects has been observed and most studies demonstrated effective results and advocated the use of conditioned media in treating disease conditions.

Specifically, conditioned media from adipose-derived mesenchymal stem cells has been used in animal models of myocardial infarction, prevention of muscular degeneration, acute and chronic hind limb ischemia, skin wound healing and alopecia. It has been shown that mesenchymal stem cells conditioned media is sufficient

World Academy of Anti-aging and Regenerative Medicine, Uchisaiwaicho, Chiyoda-ku, Tokyo, Japan and
BioMimetics Sympathies, Inc., Aomi, Koto-ku, Tokyo, Japan
* Corresponding author: M. Ghazizadeh, MD (mghazizadeh1@gmail.com).

Table 1. Conditioned media of mesenchymal stem cells

Condition	Source	Reference
Lung injury	BMMSCs	［lonuescu L,2012］
Myocardial infarction	**ADSCs**	［**Timmers L,2007**］
Cerebral injury/ischemia/stroke	BMMSCs	［Chang CP,2013］
Spinal cord injury	BMMSCs	［Yang D,2013］
Prevention of muscular degeneration	**ADSCs**	［**Servivas N,2016**］
Acute and chronic hind limb ischemia	**ADSCs**	［**Bhang SH,2014**］
Skin wound healing	**ADSCs**	［**Zhou BR,2013**］
Colitis	Amniotic fluid	［Legaki E,2016］
Acute liver injury/failure	Amniotic fluid	［Zagoura DS,2012］
Alzheimer’ s disease	DPSCs	［Mita T,2015］
Bone defects	BMMSCs	［Katagiri W,2017］
Osteoarthritis	WJMSCs	［Hassan Famian M,2017］
Corneal epithelial wound healing	hUCESCs	［Bermudez MA,2015］
Uveitis	hUCESCs	［Bermudez MA,2016］
Alopecia	**hUCESCs**	［**Park BS,2010**］
Liver fibrosis	UCPVCs	［Jang YJ,2017］
Parkinson’ s disease	WJMSCs	［Teixeira FG,2017］
Multiple esclerosis	PDLSCs	［Rajan TS,2016］
Regeneration of atrophied muscles	UCPVCs	［Kim MJ,2016］
Cancer	hUCESCs	［Eiro N,2014］

Table 2. Immunomodulation/anti-inflammatory activity

Tumor associated macrophages(TAMs)inhibit anti-tumor immunity
Anti-inflammatory cytokines:
Tumor necrosis factor 1(TGF1),Interleukin(IL)13,IL18binding protein (IL18BP),Ciliary neurotrophic factor(CNTF),Neurotrophin3(NT-3)factor IL10,IL12p70,IL17E,IL27orIL1 receptor antagonist(IL1RA)
Pro-inflammatory cytokines:
Il β ,IL6,IL8,IL9
The balance between these anti-inflammatory and pro-inflammatory cytokines will determine the final effect.
Mouse colitis model
Reduced IL6,IL8,TNF and MIP-1 pro-inflammatory cytokines
Increased IL10 anti-inflammatory cytokine
Reduced infiltration of leucocytes in ocular tissues

to significantly improve several biomarkers of diseases, and in general, to be as effective as transplantation of the corresponding mesenchymal stem cells.

Mechanisms of action of conditioned media from mesenchymal stem cells include immunomodulation and anti-inflammatory activity, anti-apoptotic activity, wound healing and tissue repair activity, neuroprotective and neurotrophic effects, angiogenesis regulation, and finally anti-tumor effect.

Tumor associated macrophages (TAMs) are a relevant inhibitor of anti-tumor immunity and a crucial barrier to successful immunotherapy. Studies have shown an inhibitory effect of the conditioned media from mesenchymal stem cells on monocyte–macrophage differentiation as well as on macrophage–monocyte de-differentiation.

It is well established that the anti-inflammatory effect of mesenchymal stem cell conditioned media is at least in part mediated by soluble immunoregulatory mole-

cules. Several of the anti-inflammatory cytokines and pro-inflammatory cytokines are listed in Table 2, and the balance between

these two groups of cytokines may determine the final effect.

In a mouse model of colitis, it was found that conditioned media treatment significantly reduced mRNA expression of pro-inflammatory cytokines, and increased mRNA expression of anti-inflammatory cytokines. It also reduced the infiltration of leucocytes in ocular tissues.

Regarding apoptosis, conditioned media from mesenchymal stem cells has shown a different effect on normal cells than on cancer cells. While an anti-apoptotic effect was observed in normal cells, however in cancer cells the conditioned media induced apoptosis both in vitro and in vivo.

On the wound healing and tissue repair, conditioned media from mesenchymal stem cells improved epithelial regeneration and especially in eye corneal injury repair, it did not produce vascularization which was good for preserving optical transparency. It also reduced macrophage inflammatory protein 1 (MIP-1) and TNF mRNA exression and had anti-fibrotic effect that can reduce scar formation.

Regarding the neuroprotective and neurotrophic effects, several studies in animal models have supported such effects attributing it to the neurotrophic factors in the conditioned media. For example, intranasal administration in a mouse model of Alzheimer’ s disease improved cognitive function and induced neuro-regeneration by reducing pro-inflammatory response by amyloid plaques.

With regard to the regulation of angiogenesis, as it is known angiogenesis is the process by which new vasculature grows from sprouts of pre-existing blood vessels. Normal angiogenesis is important during wound healing process.

Various studies have demonstrated the effect of conditioned media from mesenchymal stem cells on key steps in angiogenesis. For example, conditioned media from adipose, bone marrow and umbilical vein stem cells induce proliferation and migration of endothelial cells, promoting tube formation, as well as preventing endothelial cell apoptosis in vitro.

A number of angiogenic stimulators and inhibitors have been identified in conditioned media including TGF which had the ability to increase several growth factors such as VEGF, HGF, PDGF, IL6 and IL8. Also, conditioned media from mesenchymal stem cells treated with TGF induced blood vessel growth in an in vivo assay.

As for the anti-tumor effects of conditioned media from mesenchymal stem cells, controversial results have been reported. It appears that the effect of conditioned media from mesenchymal stem cells could depend on their tissue origin and also on the type of tumors to be treated.

For example, conditioned media from bone marrow mesenchymal stem cells had anti-tumor effect on non-small cell lung cancer but stimulatory effects on myeloma tumor cells.

In general, depending on the composition of the conditioned media of stem cells from different tissue sources, different effects are expected.

A suitable conditioned media for cancer therapy would contain low levels of factors promoting cancer progression and high levels of factors inhibiting cancer progression as listed here.

Therefore, composition of conditioned media from mesenchymal stem cells has to be quantitatively analyzed and standardized before clinical application in cancer therapy.

Clinical studies using conditioned media from mesenchymal stem cells are in fact very limited. In one study, application of conditioned media from allogeneic adipose-derived stem cells after ablative fractional carbon dioxide laser resurfacing on human skin showed enhanced wound healing by reducing adverse effects such as hyperpigmentation, erythema and increased trans-epidermal water loss [1].

Intradermal injection of conditioned media from adipose-derived stem cells into the scalp of alopecia patients significantly promoted hair growth in both male and female patients [2].

Similarly, a retrospective and observational study of female pattern hair loss treated with conditioned media from adipose-derived mesenchymal stem cells showed efficacy after 12 weeks by significantly increasing both hair density and thickness. None of the patients reported severe adverse reactions [3].

Finally, conditioned media from bone marrow mesenchymal stem cells has also been used safely to improve alveolar bone regeneration [4]. More clinical studies are

expected and need to be done in order to establish the therapeutic role of conditioned media from mesenchymal stem cells. The key advantages of conditioned media include:

1) Resolving several safety issues associated with the transplantation of living and proliferative cells such as immune compatibility, tumorigenicity, emboli formation and infections,

2) Conditioned media may be evaluated for safety, dosage and potency similar to conventional pharmaceutical agents,

3) Storage can be done without use of toxic cryopreservative agents,

4) It is more economical and practical for clinical use because of no need for cell collection procedures,

5) Mass-production is possible and finally,

6) Time and cost of expansion and maintenance of cultured stem cells could be significantly reduced and ready-to-use biological material could be immediately available for treatment of acute conditions such as cerebral ischemia, myocardial infarction, or severe burns.

One of the most important aspect of the mesenchymal stem cell conditioned media is its wide application potentials as categorized under different routes of administration such as intravenous injection may be used for anti-aging, inflammatory bowel disease, atopic dermatitis, and lifestyle diseases. Subcutaneous injection may be used for face wrinkle treatment, or scalp thinning hair treatment and other applications such as injection into the knee and ankle for joint pain and treatment for impotency and erectile dysfunction or alveolar bone regeneration purposes.

Future prospects appears encouraging. Considering that several clinical trials of mesenchymal stem cells have been approved by governmental agencies, it is reasonable to expect approval of conditioned media from mesenchymal stem cells. For example, platelet-rich plasma or amniotic fluid, which are highly complex and include numerous growth factors and exosomes, still remain poorly characterized, but they are used as a regenerative therapy in wound healing and orthopedics. Also, exosomes from dendritic cells have reached clinical-trial for immuno-therapy of certain cancers.

More clinical trials using conditioned media from mesenchymal stem cells will certainly add support to its application in regenerative medicine. However, in order to establish the safety and efficacy of these products, strict regulatory measures for manufacturing and quality control and standardization protocol will be mandatory.

In conclusion, conditioned media from mesenchymal stem cells contain secretome of such cells, composing of various biologically active substances. The conditioned media has several advantages over using the living stem cells. Many experimental animal studies and a few reported human applications demonstrate that conditioned media from mesenchymal stem cell are safe and effective to significantly improve various disease conditions. So far, no serious side effects has been reported. More clinical trials using conditioned media from mesenchymal stem cells are needed to establish its application in regenerative medicine.

Conflict of Interest

The author declares no conflicts of interest.

References

1) Zhou BR, Xu Y, Guo SL, Xu Y, Wang Y, Zhu F, Permatasari F, Wu D, Yin ZQ, Luo D. The effect of conditioned media of adipose-derived stem cells on wound healing after ablative fractional carbon dioxide laser resurfacing. Biomed Res Int. 2013:519126
2) Fukuoka H, Suga H. Hair Regeneration Treatment Using Adipose-Derived Stem Cell Conditioned Medium: Follow-up With Trichograms. Eplasty 2015:15:e10.
3) Shin H, Ryu HH, Kwon O, Park BS, Jo SJ. Clinical use of conditioned media of adipose tissue-derived stem cells in female pattern hair loss: a retrospective case series study. Int J Dermatol 2015; 54:730-735.
4) Katagiri W, Osugi M, Kawai T, Hibi H. First-in-human study and clinical case reports of the alveolar bone regeneration with the secretome from human mesenchymal stem cells. Head Face Med 2016; 12:5.