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Scaffold-based synergistic enhancement of stem cell effects for therapeutic angiogenesis in critical limb ischemia: an experimental animal study

Hyung Sub Park[1,2], Geum Hee Choi[2], Tae Woo Jung[3], Taeseung Lee[1,2]

[1]Department of Surgery, Seoul National University College of Medicine, Seoul, Korea
[2]Department of Surgery, Seoul National University Bundang Hospital, Seongnam, Korea
[3]Department of Pharmacology, Chung-Ang University College of Medicine, Seoul, Korea

Purpose: Stem cell-based therapies are considered an alternative approach for critical limb ischemia (CLI) patients with limited or exhausted options, yet their clinical use is limited by the lack of sustainability and unclear mechanism of action. In this study, a substance P-conjugated scaffold was injected with mesenchymal stem cells (MSCs) into an animal model of CLI to verify whether angiogenesis could be enhanced.
Methods: A self-assembling peptide (SAP) was conjugated with substance P, known to have the ability to recruit host stem cells into the site of action. This SAP was injected with MSCs into ischemic hindlimbs of rats, and the presence of MSCs was verified by immunohistochemical (IHC) staining of MSC-specific markers at days 7, 14, and 28. The degree of angiogenesis, cell apoptosis, and fibrosis was also quantified.
Results: Substance P-conjugated SAP was able to recruit intrinsic MSCs into the ischemic site of action. When injected in combination with MSCs, the presence of both injected and recruited MSCs was found in the ischemic tissues by double IHC staining. This in turn led to a higher degree of angiogenesis, less cell apoptosis, and less tissue fibrosis compared to the other groups at all time points.
Conclusion: The combination of substance P-conjugated SAP and MSCs was able to enhance angiogenesis and tissue repair, which was achieved by the additive effect from exogenously administered and intrinsically recruited MSCs. This scaffold-based intrinsic recruitment approach could be a viable option to enhance the therapeutic effects in patients with CLI.
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Key Words: Angiogenesis, Chronic limb-threatening ischemia, Mesenchymal stem cells, Scaffold, Substance P

INTRODUCTION

Ischemia caused by the occlusion of arteries is the main cause of cardiovascular diseases, stroke, and peripheral vascular diseases, which are known to be the leading causes of death and a major health-related issue worldwide [1,2]. In the field of peripheral arterial diseases (PAD), arterial occlusion is treated by either surgery or endovascular intervention, yet it is associated with high amputation and mortality rates [3]. In around 15%–40% of PAD patients, current surgical or endovascular treatment options are not feasible or exhausted, and this is referred to as no-option critical limb ischemia (CLI) [4]. Certain nonatherosclerotic vascular diseases, such as thromboangiitis obliterans (also known as Buerger disease), are

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Corresponding Author: Taeseung Lee
Department of Surgery, Seoul National University Bundang Hospital, 82 Gumi-ro 173-beongil, Bundang-gu, Seongnam 13620, Korea
Tel: +82-31-787-7092, **Fax:** +82-31-787-4078
E-mail: tslee@snubh.org
ORCID: https://orcid.org/0000-0001-6425-5924

also not amenable to current surgical/interventional therapies due to the lack of target runoff vessels. In these patients, other forms of treatment are required, and therapeutic angiogenesis has been proposed as a potential method for treatment.

Therapeutic angiogenesis involves the formation and budding of new blood vessels to improve perfusion, and many studies have shown that a wide variety of substances ranging from growth factors to endothelial progenitor cells can be used to improve ischemia in clinical settings [5,6]. Stem cells, with their pluripotent characteristics, are another good candidate for therapeutic angiogenesis, and many clinical randomized controlled trials have been recently reported showing their potential effect to improve ischemia in different vascular beds [7]. Our group has also previously shown possibilities for applying stem cells to improve ischemia and prevent aneurysmal changes in multiple vascular disease models [8,9]. Despite the large amount of research that is being done on stem cells during the past 2 decades, it has not achieved widespread clinical application; one of the reasons being that their mechanism of action is not yet well understood. Additionally, it is unclear how long these stem cells survive and how they integrate into the tissue when administered externally, eventually requiring multiple repeated injections to obtain a sustained effect. Different approaches are being used to improve stem cell survival or enhance their effects *in vivo*, and one of them involves the use of biological scaffolds [10]. These scaffolds can create a 3-dimensional (3D) microenvironment in the form of extracellular matrices, which protects the stem cells and prolongs their survival.

We have previously demonstrated that self-assembling peptides (SAP), when combined with mesenchymal stem cells (MSC), were able to improve angiogenesis *in vitro* and *in vivo* [11]. SAP consists of 8–16 amino acids with alternating hydrophilic and hydrophobic residues, where the hydrophilic portion has alternative units of positive and negative charged amino acids. This configuration allows for formation flexible nanofiber hydrogels of 7–20 nm in diameter under physiologic conditions, providing a stable 3D environment for cells and smaller particles [12,13]. The role of SAPs is not confined to prolongation of stem cell survival, but they can also be used to recruit intrinsic cells into their 3D configuration. A previous study demonstrated the ability of SAPs to recruit host MSCs into ischemic limbs *in vivo* to improve perfusion [14]. Its recruitment potential can be further enhanced by combining a functional motif in the sequence. Substance P, in particular, has been shown to promote recruitment of MSCs derived from the bone marrow to promote tissue repair [15-17].

In this study, we created an animal model of CLI and injected MSCs in combination with an SAP incorporating substance P. Through this approach, we attempted to verify whether the additional recruitment of host stem cells by SAP was able to synergistically enhance stem cell angiogenesis to prevent limb necrosis.

METHODS

Isolation and culture of rat mesenchymal stem cell

MSCs were isolated directly from the femoral bones of 3–4-week Sprague-Dawley male rats using a protocol previously reported [11]. In brief, cells were cultured using Dulbecco's modified Eagle's medium (Sigma Aldrich), 10% fetal bovine serum (Gibco BRL), 100-U/mL penicillin, 100-μg/mL streptomycin (Gibco BRL), and 2-mM L-glutamate (Gibco BRL) at 37 °C and 5% CO_2 atmosphere. After 3–5 passages, cells were sorted using magnetic-activated cell sorting and CD105 MultiSort kit (PE, Miltenyi Biotech) and characterized for rat-specific MSC markers CD105, CD73, CD29, and CD45. These cells were then tagged with green fluorescent protein (GFP) by transfection with a lentivirus (Macrogen).

Self-assembling peptide preparation

An SAP named RADA 16-II (Peptron), with its characteristics having been already described elsewhere, and having the configuration AcN-RARADADARARADADA-$CONH_2$ (hereinafter simplified as RADA) was used for this study [18]. Substance P was conjugated to RADA 16-II (hereinafter named as RSP) and had the configuration AcN-RARADADARARADADAGGRPKPQQFFGLM-$CONH_2$. RADA, and RSP were dissolved in sterile 295-nM sucrose at 1% (wt/vol) and sonicated for 30 minutes.

In vivo testing on a rat hindlimb ischemia model

Under the approval of the Institutional Animal Care and Use Committee of Seoul National University Bundang Hospital (No. MSRI-52-17-001), a hindlimb ischemia rat model was created in 3–4-week Sprague-Dawley rats. After isoflurane general anesthesia, a longitudinal incision was made in the left thigh of the hindlimb, and the femoral artery was identified and meticulously dissected from its surrounding structures. The femoral artery was excised entirely after ligation of the proximal and distal ends. A total of 36 rats were divided into 4 groups: MSC injection only (MSC group), RSP injection only (RSP group), combination of MSC with RADA and RSP (MSC + RSP group), and control. MSCs (1×10^7 cells) were injected intramuscularly into the thigh at 4–5 different locations during model creation (day 0) and at postoperative day 3, while for RADA and RSP, they were mixed in 200 μL of phosphate-buffered saline (PBS) and injected in the same manner. For the control group, 200 μL of PBS was injected, making sure that the same volume was injected in each group.

Immunohistochemical staining

Harvesting was performed at 7, 14, and 28 days by removal

of the thigh muscles and fixed in 10% formalin for 24 hours. After paraffin embedding and slicing (5–7 μm thickness), immunohistochemical (IHC) analysis was performed for MSC markers CD105, CD90, and CD29 by using their respective antibodies: anti-CD105/endoglin antibody (Abcam), anti-CD90/Thy1 antibody (Abcam) and anti-CD29/integrin beta-1 antibody (Abcam). IHC staining was also performed for angiogenesis markers von Willebrand factor (vWF 1:100, Dako), CD31 (1:200, Abcam), and α-smooth muscle actin (α-SMA 1:200, Abcam) using the respective primary polyclonal antibodies at 4 °C overnight and goat anti-rabbit immunoglobulin G (rhodamine, Abcam) as secondary antibody at 4 °C for 3 hours. Nuclei counterstaining was performed with 4',6-diamidino-2-phenylindole (DAPI, Vector Laboratories). Capillary density was measured by counting the positive stains at 6 different random fields 3 times at 100× magnification.

Apoptosis and fibrosis

The terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick end labeling (TUNEL) method was used to measure apoptosis. A TUNEL assay kit (Millipore) was used for staining the harvested tissues at days 7, 14, and 28, and the apoptosis ratio was calculated as the number of TUNEL-positive nuclei divided by the total number of nuclei at 5

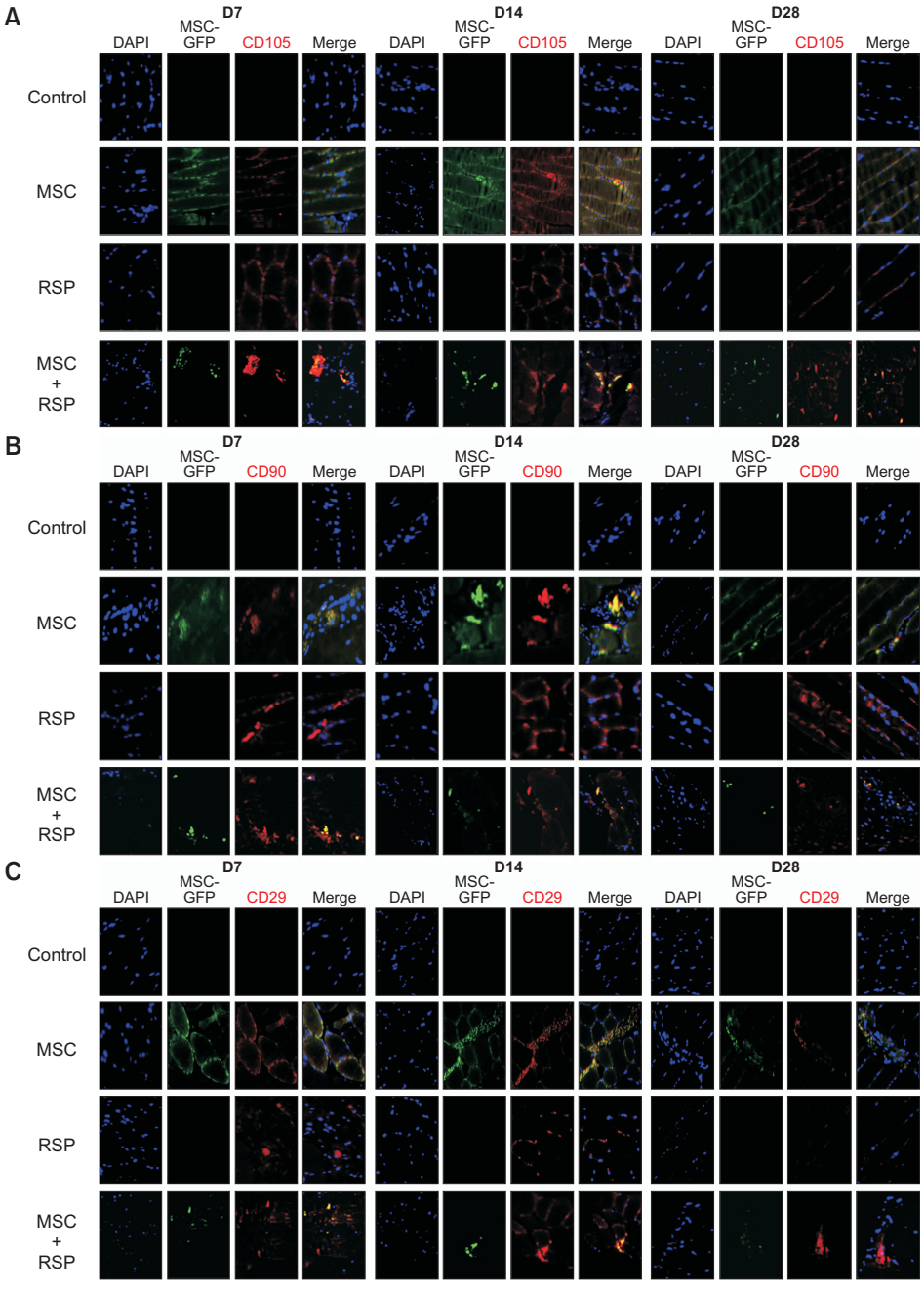

Fig. 1. Fluorescent immunohistochemical staining for stem cell markers (A) CD105, (B) CD90, and (C) CD29 from ischemic hindlimbs injected with rat MSC (tagged with green fluorescent protein), RSP, and a combination of MSC and RSP (10× magnification). MSC, mesenchymal stem cell; RSP, substance P conjugated to RADA 16-II; DAPI, 4′,6-diamidino-2-phenylindole.

different random fields (200× magnification) using a Mantra quantitative pathology imaging system (Perkin Elmer).

The degree of fibrosis formation was analyzed by staining with Masson's trichrome stain and calculated as the area of fibrosis (per mm^2) in 10 different random fields (100× magnification) using a Mantra quantitative pathology imaging system (Perkin Elmer).

Statistical analysis

All continuous variables are presented as mean ± standard error of the mean. Statistical analysis between multiple comparison groups was done by one-way analysis of variance with *post hoc* testing using the Tukey method. IBM SPSS Statistics ver. 20 (IBM Corp.) was used for analysis, and differences were considered statistically significant when $P < 0.05$.

RESULTS

RSP recruits endogenous mesenchymal stem cells to rat ischemic hindlimbs

IHC staining of tissues harvested at 7, 14, and 28 days from rat ischemic hindlimbs for detection of MSCs are shown in Fig 1. Injected MSCs were detected in the MSC group, as

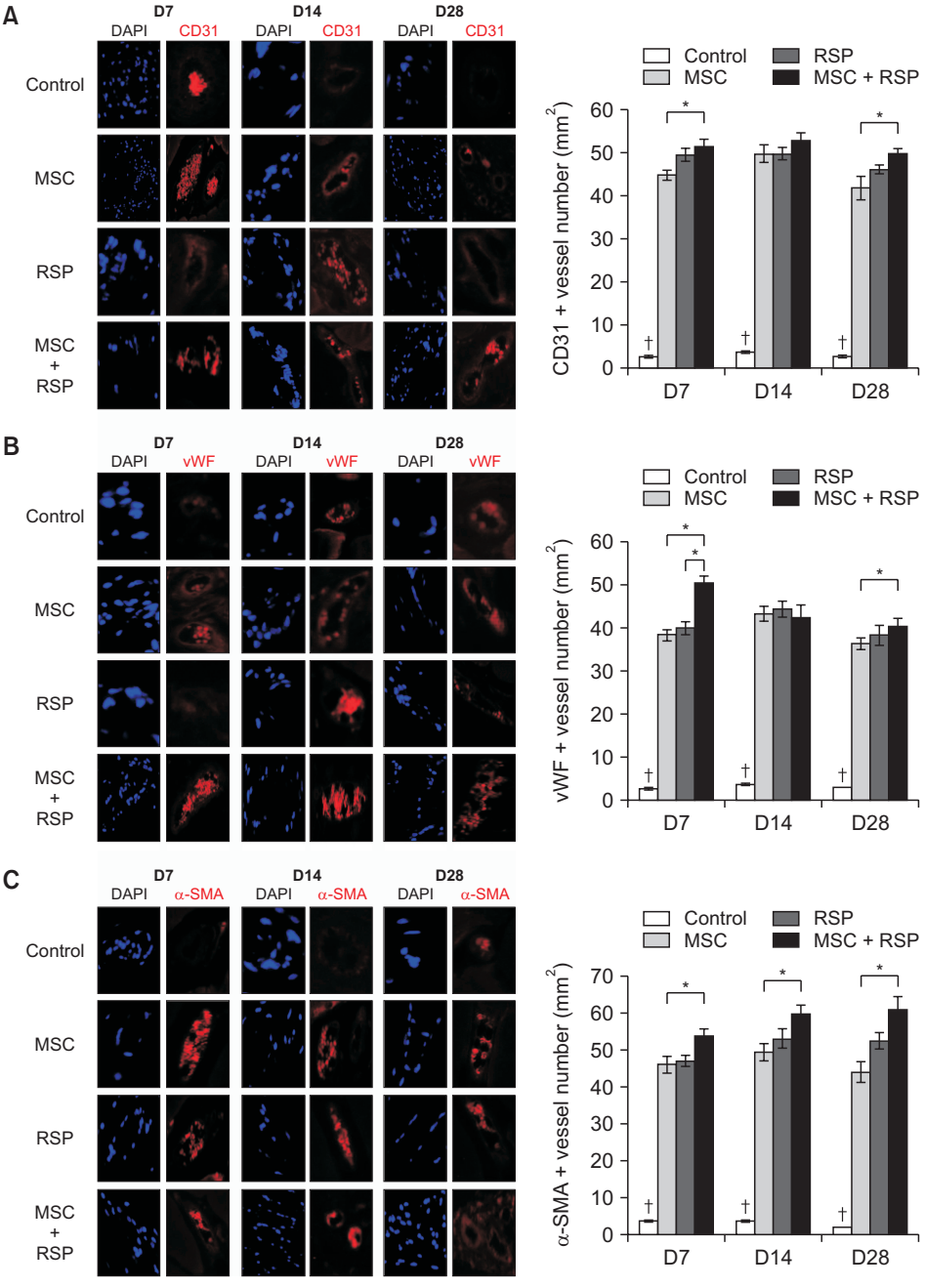

Fig. 2. Immunohistochemical staining and quantification of angiogenesis markers for MSC, RSP, MSC + RSP, and control. (A) CD31. (B) von Willebrand factor (vWF). (C) α-smooth muscle actin (α-SMA). MSC, mesenchymal stem cell; RSP, substance P conjugated to RADA 16-II; DAPI, 4′,6-diamidino-2-phenylindole. *$P < 0.05$. †$P < 0.05$ for control *vs.* all other groups.

shown by positive stains in green due to the presence of GFP, and in red for MSC-specific markers CD105, CD90, and CD29, at the different time points (days 7, 14, and 28). In the RSP group, in which no exogenous MSCs were injected (as shown by the negative stain for GFP), positive stains were seen for CD105, CD90, and CD29 at the different time points, which demonstrates that MSCs are recruited to the site of action and remain up to 28 days in the ischemic tissues. In the MSC + RSP group, the presence of all MSCs can be seen in red, while exogenously injected MSCs are stained in green. Therefore, when the 2 stains are merged, the yellow stains refer to the exogenously injected MSCs, while the remaining red stains represent MSCs that have been endogenously recruited by RSP. The merged images show a combination of yellow and red colored stains for the different MSC-specific markers across the different time points, demonstrating the presence of both injected and recruited MSCs in the ischemic tissues. Finally, the control group did not show any positive stain for all MSC markers, showing that endogenous recruitment of MSCs was related to the presence of RSP and not by the induction of ischemia alone.

Improvement in angiogenesis by RSP

IHC staining of tissues harvested at 7, 14, and 28 days for angiogenesis markers CD31, vWF, and α-SMA was performed (Fig. 2). The results demonstrate that in comparison to the control group, the MSC, RSP, and MSC + RSP groups showed a significantly increased expression of angiogenic markers at days 7, 14, and 28. When quantified, the MSC + RSP group showed an overall increase in number of angiogenic vessels compared to MSC or RSP alone, demonstrating that the combination of exogenously injected and endogenously recruited MSCs was able to exert a stronger angiogenic effect on ischemic tissue.

Reduction of apoptosis and fibrosis by RSP

Histologic changes were assessed by measuring the degree of apoptosis and fibrosis. TUNEL staining to demonstrate the degree of apoptosis showed that the control group had the statistically highest rate of apoptosis compared to all other groups at all time points (Fig. 3). On the other hand, the MSC + RSP combination group showed the lowest rate of apoptosis, followed by RSP and MSC groups. Additionally, apoptosis was highest at day 7 and tended to gradually lower at day 28, meaning that cell regeneration and tissue healing occurred as time progressed. A similar pattern was seen for tissue fibrosis, as shown by Masson's trichrome staining (Fig. 4), where the control group showed the highest degree of fibrosis compared to all other groups at 7, 14, and 28 days. Overall, the MSC + RSP group showed the lowest degree of fibrosis, followed by the RSP and the MSC group. These results demonstrate that the combination of MSC and RSP had the strongest effect of preventing apoptosis and fibrosis at the cellular and histological levels up to 28 days, which was possible by the combined effect of exogenously injected and endogenously recruited MSCs.

DISCUSSION

In this study, we were able to demonstrate that by conjugating substance P with an SAP, intrinsic stem cells could be recruited to the site of action in a rat model of hindlimb ischemia, which in turn led to an increase in markers associated with angiogenesis and a decrease in cell apoptosis

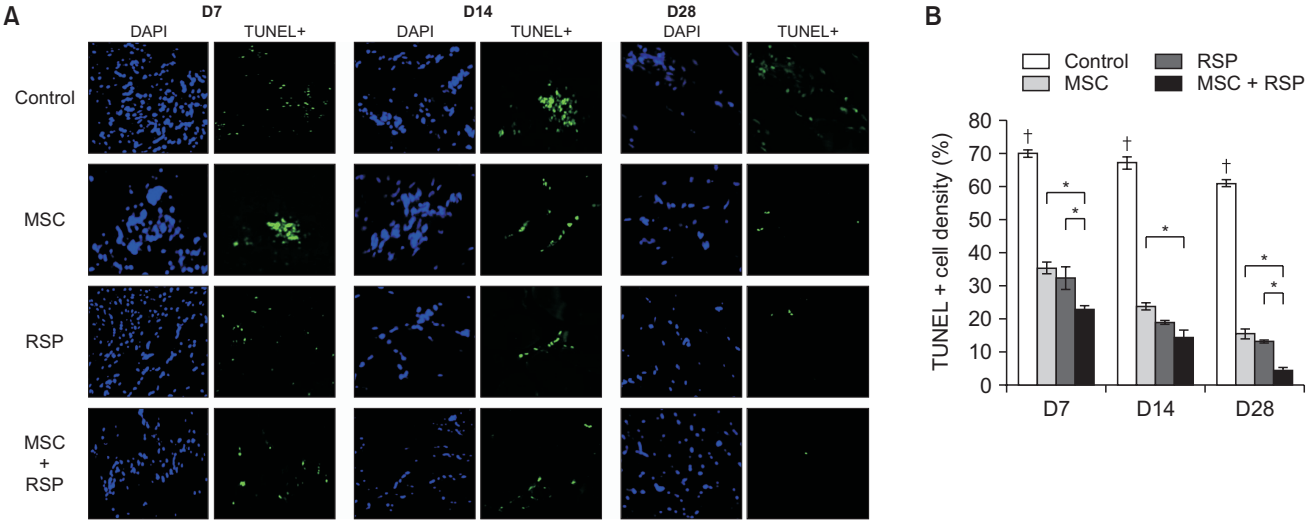

Fig. 3. (A) Fluorescent terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick end labeling (TUNEL) staining and (B) quantification of TUNEL-positive stains for evaluation of apoptosis for MSC, RSP, MSC + RSP, and control. MSC, mesenchymal stem cell; RSP, substance P conjugated to RADA 16-II; DAPI, 4′,6-diamidino-2-phenylindole. *$P < 0.05$. †$P < 0.05$ for control *vs.* all other groups.

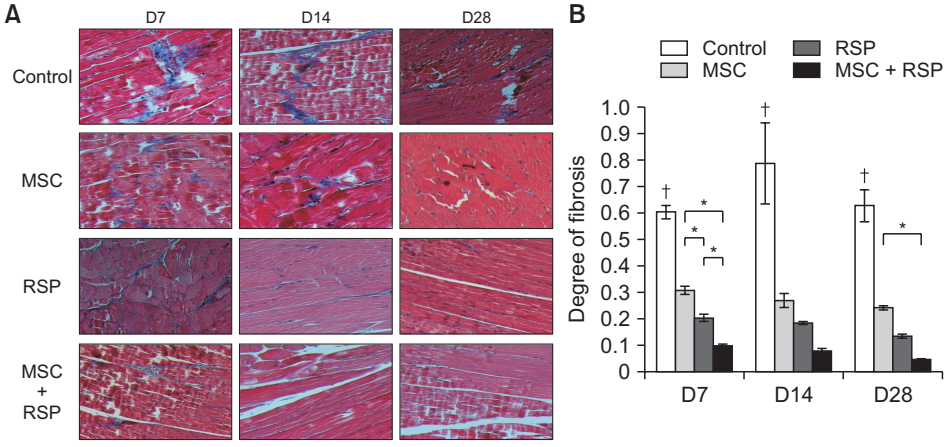

Fig. 4. (A) Representative Masson's trichrome staining showing fibrosis between muscle fibers (10× magnification) and (B) quantification of degree of fibrosis from immunohistochemical stains for evaluation of fibrosis for MSC, RSP, MSC + RSP, and control. MSC, mesenchymal stem cell; RSP, substance P conjugated to RADA 16-II. *P < 0.05. †P < 0.05 for control *vs.* all other groups.

and fibrosis. Additionally, when the substance P-conjugated SAP was combined with an additional injection of MSCs, the presence of both exogenous and recruited MSCs was verified in the ischemic tissues, which again led to higher expressions of angiogenic markers and lower rates of apoptosis and fibrosis.

Cell-based therapies have been reported as a viable alternative in no-option CLI patients, and there are many ongoing phase III trials worldwide trying to develop a medical solution for the treatment of ischemia in different tissues [19]. Despite these efforts, it is not yet being widely used in our daily practice. In a recent meta-analysis, different kinds of cell types ranging from autologous peripheral blood cells to stem cells have been analyzed, but the results were mixed; and for bone marrow MSCs, the authors concluded that there was some beneficial effect in improving perfusion parameters, though it failed to improve amputation rates [20]. The reason for this may be related to the sustainability of the effects, since despite having performed repeated injections to sustain the effects of stem cells, it may not be strong enough to regenerate broad ischemic lesions needed to reduce amputation. In this respect, recruitment of endogenous stem cells may be an important strategy for overcoming the limitations of stem cell injection therapy in clinical practice.

We previously demonstrated that the SAP RADA 16-II had the potential to recruit host MSCs into the site of ischemia in a rat hindlimb model. However, when combined with exogenously injected MSCs, its recruiting effect was diminished and primarily functioned as a scaffold for these injected MSCs [11]. In order to potentiate the recruiting effect of RADA 16-II, substance P was conjugated into the C-termini of the peptide sequence separated by a GG spacer, since previous studies have shown that the incorporation of functional peptide motifs in this location allowed for SAPs to perform specific bioactive functions related with the incorporated motif [21]. Other studies have also shown that stem cell proliferation was only enhanced after adding specific binding sequences into SAPs, while proliferation was decreased when combined with SAPs without the binding motifs [22,23]. Substance P is well-known for its role as a neurotransmitter and a modulator of pain perception, but it has many other diverse functions related to gastrointestinal functioning, vasodilation, angiogenesis, and cell growth [24-26]; and as previously mentioned, has also been shown to have a role in recruiting stem cells under certain conditions.

In this study, we were able to visualize the dual presence of injected and recruited MSCs in the ischemic lesions for up to 28 days. There were no signs of interference or competition between the different sources of MSCs and the effects were additive in terms of angiogenesis, with the combination group (MSC + RSP) showing a tendency for the highest degree of angiogenesis at different time points. Additionally, there was no decrease in angiogenic effect from day 7 to day 28, demonstrating that the effect was sustained. The resulting effects of angiogenesis were also demonstrated by the decrease in cell apoptosis and tissue fibrosis, where the combination group showed the lowest degree of apoptosis and fibrosis, with a gradual decrease from day 7 to day 28. Therefore, the conjugation of substance P to SAP allowed for additional recruitment of intrinsic stem cells to work synergistically with injected MSCs, leading to the additive angiogenic effects *in vivo*.

Our study has several limitations, including the small number of animals in each group which may have led to a lack of statistical significance in some of the results despite showing a good overall tendency. This lack of statistical significance, especially between the RSP and MSC + RSP groups in terms of angiogenesis, makes it unclear whether the effect of the combination group is truly additive or mainly from RSP alone. However, the dual presence of MSCs (injected and intrinsic) up to 28 days suggests that the effect is probably additive, and this is further complemented by a significant decrease in apoptosis and fibrosis.

In conclusion, conjugation of substance P to a SAP RADA 16-II was able to recruit intrinsic MSCs into ischemic tissues in a rat model of hindlimb ischemia. When combined with injections

of MSCs, there was an additive effect in terms of angiogenesis, which in turn led to a decrease in cellular apoptosis and tissue fibrosis. Although further studies are required for clinical applicability, this strategy of combining exogenous stem cells with an endogenous stem cell recruiting factor may be a viable option to overcome the current limitations of stem cell injection therapies in CLI patients and achieve more sustainable outcomes.

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Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

ORCID iD

Hyung Sub Park: https://orcid.org/0000-0001-8748-5925
Geum Hee Choi: https://orcid.org/0009-0001-5940-0717
Tae Woo Jung: https://orcid.org/0000-0002-1167-1892
Taeseung Lee: https://orcid.org/0000-0001-6425-5924

Author Contributions

Conceptualization: HSP, TL
Formal Analysis, Methodology: HSP, GHC
Investigation: All authors
Project Administration: TL
Writing- Original Draft: HSP
Writing- Review & Editing: All authors

REFERENCES

1. Tsao CW, Aday AW, Almarzooq ZI, Alonso A, Beaton AZ, Bittencourt MS, et al. Heart disease and stroke statistics-2022 update: a report from the American Heart Association. Circulation 2022;145:e153-639.
2. Vaduganathan M, Mensah GA, Turco JV, Fuster V, Roth GA. The global burden of cardiovascular diseases and risk: a compass for future health. J Am Coll Cardiol 2022;80:2361-71.
3. Norgren L, Hiatt WR, Dormandy JA, Nehler MR, Harris KA, Fowkes FG, et al. Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II). J Vasc Surg 2007;45 Suppl S:S5-67.
4. Sprengers RW, Teraa M, Moll FL, de Wit GA, van der Graaf Y, Verhaar MC, et al. Quality of life in patients with no-option critical limb ischemia underlines the need for new effective treatment. J Vasc Surg 2010;52:843-9.
5. Kim HJ, Jang SY, Kim JM, Kim SY, Kim BM, Kim WB, et al. Vascular endothelial growth factor (VEGF)-induced angiogenic gene therapy in a patient with critical limb ischemia. Korean J Med 2003;64:85-90.
6. Keighron C, Lyons CJ, Creane M, O'Brien T, Liew A. Recent advances in endothelial progenitor cells toward their use in clinical translation. Front Med (Lausanne) 2018;5:354.
7. Liew A, Bhattacharya V, Shaw J, Stansby G. Cell therapy for critical limb ischemia: a meta-analysis of randomized controlled trials. Angiology 2016;67:444-55.
8. Park HS, Hahn S, Choi GH, Yoo YS, Lee JY, Lee T. Muscle-derived stem cells promote angiogenesis and attenuate intimal hyperplasia in different murine vascular disease models. Stem Cells Dev 2013;22:866-77.
9. Park HS, Choi GH, Hahn S, Yoo YS, Lee JY, Lee T. Potential role of vascular smooth muscle cell-like progenitor cell therapy in the suppression of experimental abdominal aortic aneurysms. Biochem Biophys Res Commun 2013;431:326-31.
10. Zhao X, Li Q, Guo Z, Li Z. Constructing a cell microenvironment with biomaterial scaffolds for stem cell therapy. Stem Cell Res Ther 2021;12:583.
11. Park HS, Choi GH, Kim D, Jung TW, Jung IM, Chung JK, et al. Use of self-assembling peptides to enhance stem cell function for therapeutic angiogenesis. Stem Cells Int 2018;2018:4162075.
12. Zhang S, Gelain F, Zhao X. Designer self-assembling peptide nanofiber scaffolds for 3D tissue cell cultures. Semin Cancer Biol 2005;15:413-20.
13. Zhang S. Fabrication of novel biomaterials through molecular self-assembly. Nat Biotechnol 2003;21:1171-8.
14. Kim JH, Jung Y, Kim BS, Kim SH. Stem cell recruitment and angiogenesis of neuropeptide substance P coupled with self-assembling peptide nanofiber in a mouse hind limb ischemia model. Biomaterials 2013;34:1657-68.
15. Hong HS, Kim S, Jin Y, Son Y. Substance P enhances the therapeutic effect of MSCs by modulating their angiogenic potential. J Cell Mol Med 2020;24:12560-71.
16. Shafiq M, Zhang Q, Zhi D, Wang K, Kong D, Kim DH, et al. In situ blood vessel regeneration using SP (Substance P) and SDF (Stromal Cell-Derived Factor)-1α peptide eluting vascular grafts. Arterioscler Thromb Vasc Biol 2018;38:e117-34.
17. Zhang Y, An S, Hao J, Tian F, Fang X, Wang J. Systemic injection of substance p

promotes murine calvarial repair through mobilizing endogenous mesenchymal stem cells. Sci Rep 2018;8:12996.
18. Kim JH, Jung Y, Kim SH, Sun K, Choi J, Kim HC, et al. The enhancement of mature vessel formation and cardiac function in infarcted hearts using dual growth factor delivery with self-assembling peptides. Biomaterials 2011;32:6080-8.
19. Perin E, Loveland L, Caporusso J, Dove C, Motley T, Sigal F, et al. Gene therapy for diabetic foot ulcers: interim analysis of a randomised, placebo-controlled phase 3 study of VM202 (ENGENSIS), a plasmid DNA expressing two isoforms of human hepatocyte growth factor. Int Wound J 2023;20:3531-9.
20. Wahid FS, Ismail NA, Wan Jamaludin WF, Muhamad NA, Mohamad Idris MA, Lai NM. Efficacy and safety of autologous cell-based therapy in patients with no-option critical limb ischaemia: a meta-analysis. Curr Stem Cell Res Ther 2018;13:265-83.
21. Yanlian Y, Ulung K, Xiumei W, Horii A, Yokoi H, Shuguang Z. Designer self-assembling peptide nanomaterials. Nano Today 2009;4:193-210.
22. Liu X, Wang X, Wang X, Ren H, He J, Qiao L, et al. Functionalized self-assembling peptide nanofiber hydrogels mimic stem cell niche to control human adipose stem cell behavior in vitro. Acta Biomater 2013;9:6798-805.
23. Stevenson MD, Piristine H, Hogrebe NJ, Nocera TM, Boehm MW, Reen RK, et al. A self-assembling peptide matrix used to control stiffness and binding site density supports the formation of microvascular networks in three dimensions. Acta Biomater 2013;9:7651-61.
24. Mistrova E, Kruzliak P, Chottova Dvorakova M. Role of substance P in the cardiovascular system. Neuropeptides 2016;58:41-51.
25. Steinhoff MS, von Mentzer B, Geppetti P, Pothoulakis C, Bunnett NW. Tachykinins and their receptors: contributions to physiological control and the mechanisms of disease. Physiol Rev 2014;94:265-301.
26. Garcia-Recio S, Gascón P. Biological and pharmacological aspects of the NK1-receptor. Biomed Res Int 2015;2015:495704.