Receptors & Clinical Investigation 2014; 1: e286. doi: 10.14800/rci.286; © 2014 by Yoshihiko Kakinuma.
http://www.smartscitech.com/index.php/rci

RESEARCH HIGHLIGHT

Nicotinic and non-nicotinic receptor-mediated mechanisms responsible for anti-atrophy effects in muscle

Yoshihiko Kakinuma

Department of Physiology, Nippon Medical School Graduate School of Medicine, Bunkyo-ku, Tokyo, Japan

Correspondence: Yoshihiko Kakinuma
E-mail: k12417853@nms.ac.jp
Received: August 04, 2014
Published online: September 22, 2014

To cite this article: Yoshihiko Kakinuma. TNicotinic and non-nicotinic receptor-mediated mechanisms responsible for anti-atrophy effects in muscle. Receptor Clin Invest 2014; 1: e286. doi: 10.14800/rci.286.

Recently, cholinergic modulation of immune cells has drawn particular interests from researchers in clinical fields, which may lead to a breakthrough that produces a novel therapeutic modality. Other than the immunological aspects, cholinergic modulation may also provide clues for accelerating angiogenesis and preventing any associated muscle atrophy. In this Research highlight, we discuss our recent findings in the context of research advancements, focusing on nicotinic and non-nicotinic receptor-mediated anti-muscular atrophy effects.

Recent developments in medical therapeutics have promoted longevity, allowing patients to survive longer and live to older ages. During the aging process, even in the absence of distinctive diseases, the elderly can develop muscle atrophy because of progressive atherosclerosis or decreased opportunities for walking. Moreover, patients that smoke heavily or have hypertension, diabetes mellitus (DM), and hyperlipidemia, have increased susceptibility to peripheral artery disease (PAD) that can be associated with severe muscle atrophy. Regardless of the etiology, muscle atrophy is a crucial issue for quality of life (QOL) and medical economics. Indeed, muscle atrophy further interferes with the mobility of patients with PAD and can ultimately result in amputation, causing poor QOL. Therefore, maintaining muscle mass against progressive atrophy should be essential to ensure that the elderly can live well, without disturbance of their QOL.

To accelerate angiogenesis and maintain muscle mass in patients with PAD, numerous therapeutic modalities have been investigated and applied. However, outcomes have been less than expected, particularly for patients with uncontrolled atherosclerosis or DM. Present therapeutic modalities include drugs (e.g., anti-platelets such as cilostazol and prostacyclin) and a regenerative therapy (e.g., cell therapy using bone marrow cells or endothelial progenitor cells). A reason for treatment failure is that PAD and severe muscle atrophy in these patients is often resistant to intervention.

These findings suggest that some factors derived from the skeletal muscle microenvironments may be prerequisites for efficient angiogenesis. This concept has been supported in another study, in which angiogenesis in the skeletal muscle of interleukin (IL)-1β-knockout mouse was significantly impaired despite transfusion with IL-1β-intact endothelial progenitor cells [1]. They demonstrated that the model was resistant to cell therapy with an impaired response caused by reduced cytokine production

from the recipient muscle, but not from the donor cells [1]. Similarly, we previously demonstrated that rat cardiomyocytes, H9C2 cells secreted vascular endothelial growth factor (VEGF) and that the culture medium with those angiogenic factors accelerated in vitro angiogenesis (i.e., a tube formation) [2]. This suggests that muscle cells play a role in accelerating angiogenesis by producing angiogenic factors [2].

From another perspective, endothelial cells in skeletal muscle are reported to be anatomically located very closely to satellite cells [3]. Thus, satellite cells (i.e., candidate skeletal muscle stem cells) are close to capillary endothelial cells and can interact through the production of certain factors. These cells, normally quiescent in skeletal muscle, will be activated by triggers such as myotrauma, accelerate myogenesis (i.e., proliferation and self-renewal), and finally become differentiated. These facts suggest that satellite cells may be crucial to the physiological response of factor secretion in angiogenesis, affecting endothelial cells in the skeletal muscle.

Our previous study demonstrated that the anti-Alzheimer drug donepezil, which is an acetylcholinesterase inhibitor (AChEI), potentiates the acceleration of angiogenesis, when we focused on endothelial cells [4]. However, VEGF-positive cells other than endothelial cells were also recognized to be induced by donepezil. The novel anti-muscle atrophy effect of donepezil observed in that study is noteworthy, which demonstrated that donepezil-treated mice showed faster recovery from muscle loss within 1–2 weeks after hindlimb ischemia, when compared with non-treated mice. This suggests that the effects of donepezil appear initially in the skeletal muscle, which may be the primary target of donepezil, followed by endothelial cells because angiogenesis cannot be accelerated within 1–2 weeks. However, this issue was left unresolved and was not completely investigated in that study. Instead, it has been recently investigated by our colleagues Noguchi T *et al*. [5] and in our recent report "Anti-muscle atrophy effect of nicotine targets muscle satellite cells partly through a α7 nicotinic receptor in a murine hindlimb ischemia model [6]". We reported that nicotine triggered angiogenic factors to be expressed, e.g., VEGF and fibroblast growth factor-2, by both cultured murine satellite cells and C2C12 cells [6]. In particular, the effect of nicotine on VEGF expression was observed with a very low nanomolar concentration of nicotine, which was clearly countered by a nicotinic receptor antagonist (mecamylamine), suggesting that nicotinic receptors were involved in the synthesis of angiogenic factors in C2C12 cells. These results confirm that either satellite cells or C2C12 cells possess an ability to produce angiogenic factors partly through nicotinic receptors.

What is the role of nicotinic receptors in those myoblast cells, e.g. satellite cells and C2C12 cells? Nicotine upregulated the phosphorylation of several cell survival signals (i.e., Akt and CREB) and alternatively downregulated markers for muscle atrophy (i.e., FOXO1, MuRF1 and mMAFbx). This suggests that nicotine activated cell survival and inhibited muscle atrophy. Furthermore, nicotine increased the gene expression of myosin heavy chain type I and IIb, indicating that nicotine helps to activate C2C12 cell hypertrophy. However, our study revealed that the effective dose of nicotine should be optimized to be within a narrow and lower nanomolar concentration. When we applied a dose over that range to mice with hindlimb ischemia, the anti-muscle atrophy became more obscure and was associated with side effects. Thus, we determined which of the α nicotinic receptors were involved in the anti-muscle atrophy effect. Our results revealed that the anti-atrophy effect of nicotine was dampened in α7 nicotinic receptor knockout (α7 KO) mice and that a α7 nicotinic receptor agonist (PNU282987) then salvaged muscle mass subjected to hindlimb ischemia. However, although the α7 nicotinic receptors appear responsible for the anti-atrophy effects observed, we cannot exclude other nicotinic receptors.

In terms of a potential clinical application for our data, we considered that nicotine should be hesitated to be prescribed for patients. Therefore, we instead investigated the effects of galantamine on anti-muscle atrophy in comparison with donepezil [6]. Galantamine has also been prescribed to patients with Alzheimer's disease and functions as an AChEI that acts through nicotinic receptors as an allosteric potentiating ligand. As observed in mice treated with nicotine, galantamine suppressed ischemia-induced hindlimb muscle atrophy. This was also accompanied with increases in VEGF and paired box protein Pax7 immunoreactivities in the satellite cells of wild-type mice over a 2-week treatment period. Moreover, galantamine surprisingly blunted the progression of muscle atrophy induced by hindlimb ischemia, even in the α7 KO mice. First, these results suggest that the α7 nicotinic receptors are not the only receptors involved in the anti-muscle atrophy effect and that nicotinic receptors instead become functionally redundant to compensate other receptor subtypes. Second, they suggest that galantamine stimulates an unknown receptor, recognized by the medication. These possibilities need to be investigated in the near future.

Donepezil also raised the same issue as galantamine because it suppressed muscle atrophy, even in α7 KO mice [4]. The anti-muscle atrophy effect of donepezil was not blunted by any nicotinic and muscarinic receptor

antagonists during the thermographic and muscle mass laterality analyses. The data using donepezil suggested that it requires unknown receptors to execute its anti-atrophy effect. The molecular structures obviously differ between galantamine and donepezil. However, the unexpectedly similar phenotypes of the different AChEIs might provide a novel but common conceptual clue to the mechanisms underlying the anti-atrophy effects that are independent of the known nicotinic receptors.

How could the potency difference in the anti-atrophy effects be determined between donepezil and galantamine? In our studies [5, 6], we did not extensively study the dose-dependent effects of either donepezil or galantamine. Nevertheless, we selected an often-reported dose of galantamine (i.e., 1 mg/kg/day), and a much lower than reported dose of donepezil (i.e., 0.2 mg/kg/dose). Compared with galantamine, even at a lower dose, donepezil demonstrated distinctive effects on hindlimb muscle mass laterality, i.e., the left to right skeletal muscle mass weight evaluated by computed tomography [5]. The laterality in the donepezil-treated mice, which was initially below 1.0, increased to over 1.0 shortly after treatment and continued at the level during follow-up. However, galantamine did not increase the laterality above 1.0, although its anti-atrophy effects were identified. These findings in donepezil-treated mice with hindlimb ischemia were striking and represent a unique aspect of donepezil. However, in both studies, we did not closely evaluate whether donepezil-treated muscles possess functional advantages over galantamine-treated ones. Therefore, a comparative study is needed.

Our present study and others [4-6] support the possibility of pharmacological intervention against muscle atrophy with cholinergic modifications, and the possibility of AChEIs as adjunctive therapy to enhance the effects of physiotherapy or rehabilitation in patients with muscle atrophy.

Conflict of interest

The authors have declared that no competing interests exist.

References

1. Tateno K, Minamino T, Toko H, Akazawa H, Shimizu N, Takeda S, *et al*. Critical roles of muscle-secreted angiogenic factors in therapeutic neovascularization. Circ Res 2006; 98:1194-1202.
2. Kuwabara M, Kakinuma Y, Ando M, Katare GR, Yamasaki F, Doi Y, *et al*. Nitric Oxide Stimulates Vascular Endothelial Growth Factor Production in Cardiomyocytes involved in angiogenesis. J Physiol Sci 2006; 56:95-101.
3. Christov C, Chrétien F, Abou-Khalil R, Bassez G, Vallet G, Authier FJ, *et al*. Muscle satellite cells and endothelial cells: close neighbors and privileged partners. Mol Biol Cell 2007; 18:1397-1409.
4. Kakinuma Y, Furihata M, Akiyama T, Arikawa M, Handa T, Katare RG, *et al*. Donepezil, an acetylcholinesterase inhibitor against Alzheimer's dementia, promotes angiogenesis in an ischemic hindlimb model. J Mol Cell Cardiol 2010; 48:680-693.
5. Noguchi T, Kakinuma Y, Arikawa M, Okazaki K, Hoshino E, Iiyama T, *et al*. Donepezil can improve ischemic muscle atrophy by activating angiomyogenic properties of satellite cells. Circulation J 2014; 78: 2317-2324.
6. Kakinuma Y, Noguchi T, Okazaki K, Oikawa S, Iketani M, Kurabayashi M, *et al*. Antimuscle atrophy effect of nicotine targets muscle satellite cells partly through an α7 nicotinic receptor in a murine hindlimb ischemia model. Transl Res 2014; 164: 32-45.