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Research Article

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Internalization of IGF-I into Skeletal Muscle Cells

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ABSTRACT

Background: IGF-I is a growth factor with known developmental and growth modulating functions on skeletal muscles. E-peptides, either EA or EB peptides, are the C terminal part of the IGF-I growth factor. AIM: To compare the differences between EA and EB internalization of IGF-I into skeletal muscle cells. Method:C2C12 muscle cells were starved overnight in serum free medium, and then treated with either IGF-I alone or IGF-I and either EA or EB synthetic peptides. The final group received no treatment, and acted as a negative control. A pilot immunofluorescence staining experiment was performed to compare IGF-I internalization in C2C12 cells. Results: The results showed more internalization of IGF-I into cells when they were treated with EA peptide compared to treatment with EB or no treatment. Conclusion: E-peptide had an effect on internalization of IGF-I into C2C12 muscle cells, and more signals were seen with EA peptide treatment, which was expressed as a change in function.

Keywords: *IGF-I; Growth factor; Skeletal muscle*

INTRODUCTION

Insulin-like growth factor-1 (IGF-I) is a growth factor with dual functions. IGF-I controls pre- and post-natal growth and development in most organs and tissues [1,2]. IGF-I is produced in the liver[3]. Some other body cells also produce their own IGF-I in response to its presence. Skeletal muscles produce and use IGF-I internally. Skeletal muscle development requires IGF-I to regulate and organize its growth. An experiment in mice showed that absence of IGF-I caused atrophy of the organs and muscle tissues; the mice's body weight was 60% lower and 95% of them died before birth. IGF-I function is important in the body for pre- and postnatal development [4].

IGF-IA and IGF-IB are two forms of IGF-I in rodents, and an E-peptide extension differentiates between them. IGF-I is available with or without the E-peptide extension. Mature IGF-I is the form without E-peptide extension and pro-IGF-I is the form with E-peptide[5]. Mature IGF-I and pro-IGF-I are normally present, and they have unique and common properties. IGF-I, IGF-IA, and IGF-IB expressions cause different levels of hypertrophy [6,7]. EB and EA peptides modulate the function of IGF-I in cells [8].

The results of previous studies showed that E-peptide can modulate the effect of IGF-I on skeletal muscle cells. The goal of this study was to compare the effect of EA and EB on IGF-I internalization in skeletal muscle cells.

MATERIAL AND METHODS

Immunofluorescence staining

We conducted a pilot study to determine if there was more internalization of IGF-I into C2C12 cells when treated with IGF-I+EA or IGF-I+EB. The Chinese hamster ovary cell line (CHO) was used, and cells were transfected with a plasmid containing the mature IGF-I epitope tagged with FLAG.A group of CHO cells that were transfected with an empty vector served as a negative control. Cells were grown on trans-well membrane plates (3413, 0.4 µm pore size;) in F12 and 10% fetal bovine serum growth medium, supplemented with 100 U/mL ampicillin and 200µg/ml G148 antibiotics. One milliliter of growth medium and 2.5×105cells were plated in each well, and they were incubated overnight to ensure attachment. On the same day, C2C12 cells were grown on coverslips (040813-9 Microscope Cover Glas. Fisherband) in 24-well plates in DMEM growth medium containing, 10% fetal bovine serum, and streptomycin + gentamicin, and were incubated overnight at37°C in an incubator to reach 50% confluence. The next day, the growth medium was removed from the C2C12 cells and replaced with differentiating media (DMEM containing 2% horse serum, and streptomycin + gentamicin). The trans-wells were transported into the wells to allow IGF-I to be released from transfected CHO cells to enter into the C2C12 cultures. One group received synthetic EA peptide at a dilution of 1:100, and the second group received synthetic EB peptide at the dilution of 1:10000 (based on GenBank AY878192 and AY878193, respectively), which were synthesized by Bio-Synthesis Inc. The third group received nothing, and the fourth group contained CHO cells that were transfected with an empty vector as a negative control. The plate was then placed in to an incubator for 24 hours. The next day, the medium was collected and stored at −80°C for future ELISA quantification of IGF-I, and the C2C12 cells were fixed on a coverslip glass using 4% formaldehyde.

RESULTS

Immunofluorescence staining

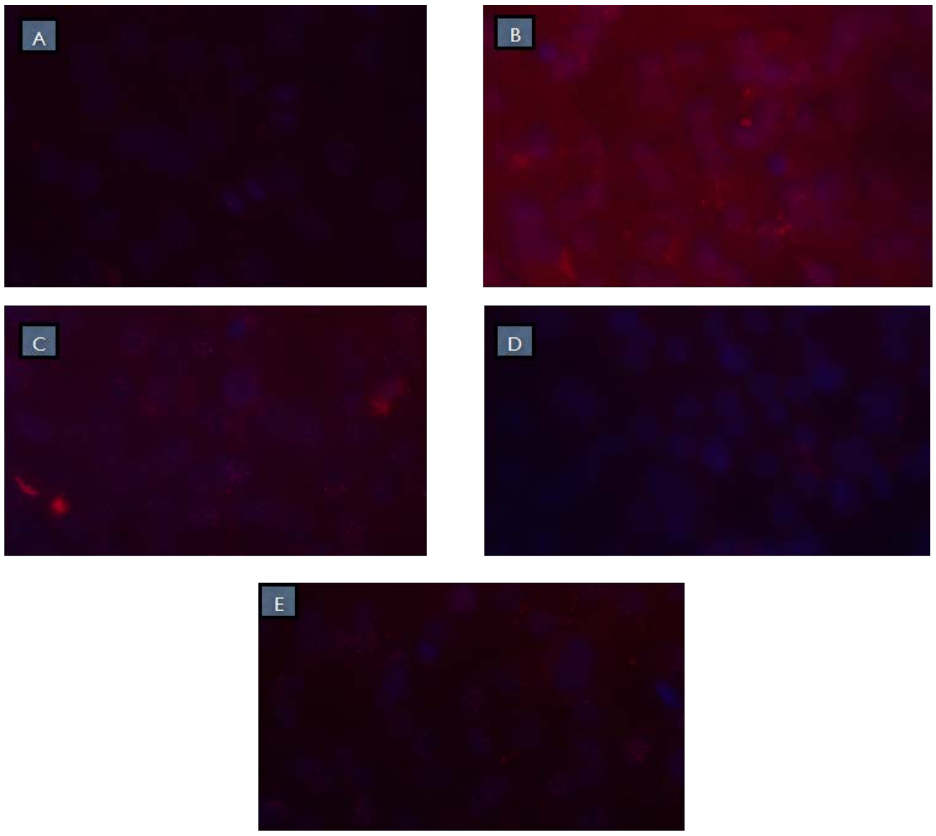

Figure 1. A: Cells treated with transfected Flagged IGF-I stop CHO cells only. B: Cells rerated with transfected Flagged IGF-I stop CHO cells plus synthetic EA peptide. C: Cells rerated with transfected Flagged IGF-I stop CHO cells plus synthetic EB peptide. E: Cells treated with empty vector transfected CHO cells.

E-peptide caused more internalization of IGF-I into cells after synthetic E-peptides were added to cells that were treated with transfected CHO cells, producing Flag-tagged mature IGF-I. Flag staining was the marker that showed IGF-I internalization and cells expressing more signals were considered to be positive for IGF-I that had entered the cell structure Fig. 1.

DISCUSSION

Studies showed that the presence of E-peptides increased IGF-I internalization into C2C12 skeletal muscle cells [9]. Studies on E-peptides suggest that they are separate growth factors that work on their own. Recently, E-peptide's dependence on IGF-I and IGF-I receptor activity has been shown in myoblast cell culture[10]. Other studies' results also showed that, when cells were treated with E-peptides alone, there was 2–4-fold increase in ERKI/II signaling compared to cells that received no treatment. EA peptide was shown to augment IGF-I potency, which is consistent with our findings. In contrast to our results, other studies found that E-peptide enhancement of IGF-IR activation did not alter the AKT/P13kinase pathway, whereas we found synthetic EA peptide added to cells treated with IGF-I significantly increased the AKT signal at 30 minutes compared to cells that received IGF-I treatment alone. We also found that EB added to IGF-I-treated cells seemed to sustain signal activation for a prolonged period of time.

Synthetic EA peptide showed increased internalization of IGF-I in C2C12 cells under immunofluorescence staining. A previous study found no statistically significant differences between cells treated with IGF-I-EA and IGF-I-EB [9]. The cells were transfected with the constructs, and there was constant production of IGF-I, and EA or EB, while in our study, the EA and EB peptides were added only at the beginning of the experiment. EB peptide has a short half-life, and its effect duration is relatively short (half-life of approximately 3 h). This experiment needs to be repeated to determine if EA's effects can be duplicated, and EB could be re-designed to block peptides' breakdown.

For the immunofluorescence experiment, quantification of the amount of IGF-I produced from CHO cells by ELISA would eliminate the possibility of extra signaling resulting from extra IGF-I produced, and vice versa. Previous studies performed in our lab using the same transfected CHO cells quantified the amount of Flagged IGF-I stop produced by these cells in each trans-well [3,9]. The amount produced was thought to be in the range of 1 nM, which was the basis for the present experiments. There should be enough IGF-I to promote signaling in the C2C12 cells, but our results should be confirmed to examine the amount of IGF-I in each well. Many investigators have targeted IGF-I therapeutic benefits in treatment of muscle diseases and damages. Studying the modulation effect of E-peptides on IGF-I might help in revealing the mechanisms of action of this growth factor, and help in understanding the biological basis of its function. One of the main questions that has not yet been answered is how the IGF-I growth factors bind to the IGF-IR, and how the E-peptide augments receptor activation. Further E-peptide investigation is vital for a complete understanding of IGF-I functions and enhancements.

CONCLUSION

More signal markers were seen in cells treated with synthetic EA peptide. Using IGF-I with the EA peptide extension caused greater IGF-I internalization into muscle cells compared to EB and IGF-I alone. Further studies are needed to quantify the effects of the differences.

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