MECHANOTRANSDUCTION PATHWAYS OF LOW INTENSITY ULTRASOUND IN CHONDROCYTES

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INTRODUCTION

Low intensity ultrasound (LIUS) is a special type of sonic pressure that can generate radiation forces, shear stresses and cavitation [**1**]. It was shown to affect the cartilage metabolism and enhance the cartilage repair. It is also being wildly applied in the cartilage tissue engineering using the 3-dimensional culture of chondrocytes and mesenchymal stem cells. In contrast to the diverse therapeutic applications of LIUS *in vitro* and in animal models, little studies have been reported regarding the precise role and cellular mechanism of LIUS in the regulation of chondrocyte function. LIUS was reported to induce aggrecan gene expression via calcium signaling in rat fibroblast and induce fibroblast proliferation via integrin/Rho/ROCK/Src/Erk signal pathways [**2**]. In general, mechanical stimuli are known to be mediated through integrins, stretch activated ion channels (SACs) and interleukin-4 (IL-4) on the chondrocyte membrane [**3**]. These signals regulate intracellular calcium levels and activity of related of signaling molecules such as protein kinase C (PKC) and mitogen activated protein kinases (MAPKs) in a diverse manner. Therefore, the effects of mechanical stimuli could be varied depending on the types of stimulation and experimental conditions; hence a precise action mechanism of mechanical stimuli should optimize the treatment condition in a particular disease context.

This study was intended to understand the mechanotransduction pathways of LIUS in association with integrins, SACs and MAPKs in chondrocytes. The relationship between these signaling pathways and the LIUS effects on the expression of matrix proteins of type II collagen and aggrecan was examined by using specific inhibitors for the signals.

MATERIALS AND METHODS

C-28/I2 cells (human chondrocyte cell line) were cultured in monolayer and treated with LIUS at an intensity of 200 mW/cm^2 using Noblelife^{TM} (Duplogen Inc., Suwon, Korea). The role of stretch-activated channels (SAC) and integrins was first examined in mediating the LIUS effects on the expression of type II collagen and aggrecan by RT-PCR and Western blot analysis. Inhibitors for SACs (gadolinium) and integrins (GRGDSP peptide or anti-integrin α1 antibody) were used to confirm their specificity. The involvement of three MPAKs signal pathways in the LIUS-mediated phenotypic changes of chondrocytes and its mechanotrnsduction pathways was also investigated using the phospho-specific antibodies.

Similar approaches are currently undergoing using rabbit primary chondrocytes in three-dimensional alginate culture.

RESULTS

Effect of LIUS on the expression of cartilage matrix proteins

C-28/I2 cells were stimulated with LIUS, and the optimal conditions for incubation and treatment times were examined in terms of type II collagen and aggrecan expression by RT-PCR. The mRNA level of type II collagen was the highest after 3 hr and that of aggrecan was gradually increasing by time, when cells were treated with LIUS for 15 min.

Role of SACs in the LIUS signal

When examined at 1, 3 and 6 hr after stimulation by RT-PCR, the LIUS effects on the mRNA levels of type II collagen and aggrecan were reduced by gadolinium treatment depending on time (Fig. 1A). When examined by immunocytochemical staining for type II collagen, the LIUS effect was also inhibited by the pre-treatment of gadolinium.

Role of integrins in the LIUS signal

C-28/I2 cells were pre-incubated with the inhibitor (GRGDSP) or a control peptide (GRADSP) for 10 min before LIUS treatment. The mRNA levels of type II collagen and aggrecan were clearly induced by LIUS in the presence of GRADSP but were lower than the untreated control (0 hr) when GRGDSP was co-treated.

MAPKs as downstream mediators of LIUS signal

The phosphorylation of ERK and JNK was induced by LIUS but that of p38 kinase was not (Fig. 2). When specific inhibitors of ERK signals (PD98059 and U0126) were pre-incubated with LIUS treatment, the LIUS-induced ERK phosphorylation was inhibited in a dose-dependent manner. Finally, pre-incubation of blocking antibody of integrin was also shown to inhibit the LIUS effect on the phosphorylation of ERK but that of pre-immune serum was not.

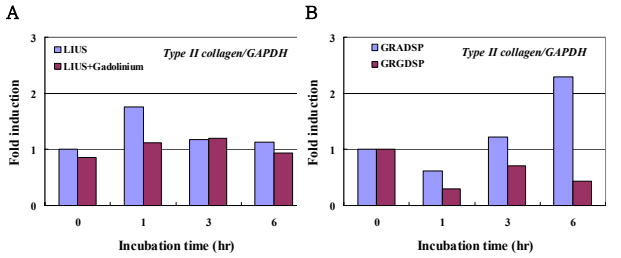

Fig. 1. Effects of gadolinium (a) and integrin inhibitor (b) on the LIUS-induced type II collagen expression. The fold induction was presented in the histogram.

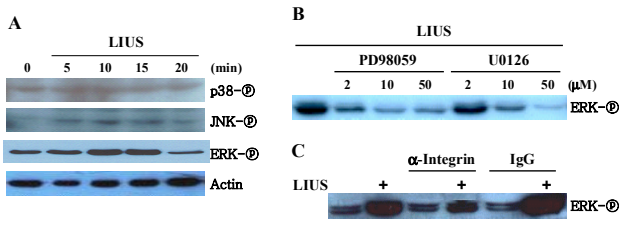

Fig. 2. Effects of LIUS on the activation of MAPKs. (a) Phosphorylation of MAPKs was measured at the indicated time points. The inhibitory effects of ERK inhibitors (b) or an integrin antibody (c) on the LIUS-induced ERK activation.

CONCLUSION

These results suggest that the LIUS signal might be mediated via canonical mechanoreceptors of SACs and integrins and subsequently through JNK and ERK pathways. Further studies are necessary to understand more details of the LIUS signaling network and regulation mechanisms. In addition, our ongoing studies in a 3-D culture of chondrocytes would give more important information about the cellular and molecular mechanism(s) of LIUS effects on development of chondrogenic phenotypes.

REFERENCES

1. **Feril, L.B. Jr. and Kondo, T.** Biological effects of low intensity ultrasound: the mechanism involved and its implications on therapy and on biosafety of ultrasound. *J. Radit. Res.*, 2004, **45**, 479-489.
2. **Zhou, S., Schmelz, A., Seufferlein, T., Li Y., Zhao, J., Bachem, MG.** Molecular mechanisms of low intensity pulsed ultrasound in human skin fibroblasts. *J. Biol. Chem.*, 2004, **279**, 54463-54469.
3. **Salter, D.M., Millward-Sadler, S.J., Nuki, G. and Wright, M.O.** Integrin-interleukin-4 mechanotransduction pathways in human chondrocytes. *Clin. Orthop. Relat. Res.*, 2001, **391S**, S49-60.

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