MECHANICAL STRESS ACTIVATES LYSOPHOSPHATIDIC ACID RECEPTOR WITHOUT THE INVOLVEMENT OF LYSOPHOSPHATIDIC ACID IN HUMAN BLADDER SMOOTH MUSCLE CELLS

Hypothesis / aims of study
Lysophosphatidic acid (LPA) is a bioactive lipid that influences a wide range of biological processes, including cell proliferation, migration, morphologic changes, and survival. However, a role for LPA in bladder pathology remains to be elucidated. Bladder smooth muscle hyperplasia is a part of the usual compensatory response to bladder outlet obstruction (BOO) resulting often from benign prostatic hyperplasia (BPH). Since, mechanical stretch stress is considered the trigger inducing smooth muscle hyperplasia secondary to BOO, we used an in vitro model of mechanical stress and showed that mechanical stress activated c-Jun NH2 terminal kinase (JNK) in human bladder smooth muscle cells (HBSMCs), which is a member of mitogen activated protein kinase (MAPK) (1). In the present study, we first detected the expression of LPA receptors in HBSMCs. We also investigated whether LPA receptors are involved in stretch-induced JNK activation.

Study design, materials and methods
In all experiments, we used commercially established HBSMCs purchased from Lonza. We performed real-time reverse-transcription PCR (RT-PCR) analysis to determine mRNA expression levels of the LPA receptors in HBSMCs. After HBSMCs were treated by LPA or mechanical stretch for 15 min, the activity of JNK was measured by western blotting method using anti-phosho JNK antibody. To simplify the analysis, we used uni-axial stretch device (STREX) which can stimulate adherent cells to a single direction stretch by controlled motor unit (Fig. 1). Cells were stimulated by 15% elongated uni-axial stretch at 0.5 Hz. To investigate whether LPA is secreted from HBSMCs into the culture medium by stretch, HBSMCs were exposed to conditioned medium collected after stretching HBSMCs for 15 min or to the indicated concentrations of LPA (10^-8-10^-5 M), and we compared JNK activation induced by them. All values are expressed as means ±SE. The data were statistically analysed by one-way ANOVA with the Dunnett’s test, and a probability value of p < 0.05 was considered significant.

Results
LPA1 mRNA was predominantly expressed in HBSMCs (Fig. 2). In HBSMCs, LPA solely induced JNK activation by dose-dependent manner (Fig. 3), and this activation was completely suppressed by pre-incubation with LPA1/3 inhibitor Ki16425 (Fig. 4). Mechanical stretch (15% elongation, 0.5 Hz) activated JNK, and this activation was also inhibited by pre-incubation with Ki16425 (Fig. 4). The medium conditioned by stretching HBSMCs for 15 min did not activate JNK (Fig. 3).

Interpretation of results
The results showed that LPA1 receptor may be expressed predominantly in HBSMCs, and that LPA activates JNK through LPA1 receptor. Mechanical stretch also activated JNK, which was shown to be suppressed by the LPA receptor antagonist (Ki16425). Since, the medium conditioned by stretching HBSMCs never caused JNK activation, it is not likely that LPA was released from HBSMCs by mechanical stretch (autocrine fashion). These results suggest that Ki16425 works as an inverse agonist of the LPA receptor. Thus, mechanical stress may directly activate the LPA receptor without involvement of LPA, thereby activating the downstream pathway (JNK).

Concluding message
Clinical implication from the present study is that LPA1 receptor antagonist having an inverse agonistic action may prevent the development of bladder smooth muscle hyperplasia secondary to BOO.
Fig 1. Schema of uni-axial stretch device

Fig 2. Expression of LPA receptor subtype in HBSMCs. (n=10)
The results were expressed as the relative ratio to beta-actin mRNA expression, the data of LPA1 receptor mRNA expression was set at 1.

Fig 3. Activation of JNK by LPA and conditioned medium. (n=5)
HBSMCs were exposed to conditioned medium collected before (-) or after stretching (st) HBSMCs for 15 min or to the indicated concentrations of LPA (10^{-8}-10^{-5} M). *p<0.05 vs. (-)

Fig 4. Activation of JNK in HBSMCs, pretreated with 10^{-6} M Ki16425 or vehicle (-), were stimulated with 10^{-5} M LPA or mechanical stretch for 15 min. Activation of JNK was determined by using antibody against phosphorylated JNK.

References

Specify source of funding or grant

Is this a clinical trial?
No
| What were the subjects in the study? | NONE |