Inhibitors of Mitogen-activated Protein Kinase Kinase Potentiated Agonist-induced Airway Smooth Muscle Contraction

Loh S.C. 1, Alan Koh H.M. 2 and Fred Wong W.S. 3

Department of Pharmacology, Faculty of Medicine, National University of Singapore, MD2 18 Medical Drive, Singapore 119260.

ABSTRACT

PD 098059 (2’-amino-3’methoxyflavone) has been shown to inhibit the activation of mitogen-activated protein kinase kinase-1 (MAPKK 1) by Raf-1 or mitogen activated and extracellular regulated kinase kinase (MEK kinase) in vitro while U0126 [1,4-diamino-2,3-dicyano-1,4-bis(2-aminophenylthio)butadiene] was found to functionally antagonized AP-1 transcriptional activity via noncompetitive inhibition of dual specificity kinase, MEK 1 and MEK 2. The present study was conducted to examine the potentiation effects of these two MAPKK inhibitors, PD 098059 and U0126, on agonist-induced bronchial smooth muscle contraction. PD 098059 (10–50µM) potentiated both histamine- and leukotriene D4 (LTD4)-induced bronchial smooth muscle contraction. 30µM of U0126 has potentiated the LTD4-induced bronchial smooth muscle contraction, as well as that by histamine. Our data show that MAPKK inhibitors can potentiate agonist-induced airway smooth muscle contraction in vitro, probably via the inhibition of relaxant prostanoids production.

INTRODUCTION

MAPKK is part of the Ras/MAPK signaling pathway that engages in various activities such as metabolic processes, cell cycles, cell migration, cell shape, cell proliferation as well as cell differentiation. PD 098059 and U0126 (Fig. 1) are two well-known specific MAPKK inhibitors that have been used to study the importance of this pathway (Alessi et al., 1995; Favata et al., 1998). Histamine and LTD4 are the two main mediators that cause bronchoconstriction by mediating a certain degree of tone in airway smooth muscle in vitro (Ellis and Undem, 1994). Our previous study showed that histamine-induced bronchial contraction was significantly potentiated by PD 098059 (Tsang et al., 1998). In the present study, we examined the potentiation effects of PD 098059 and U0126 on agonist-induced bronchial smooth muscle contraction. Our findings show PD 098059 (10–50µM) and U0126 (30µM) potentiated both histamine- and LTD4-induced bronchial smooth muscle contractions. This potentiation effect is likely due to the inhibition of relaxant prostanoids production in the airways by PD 098059 and U0126.
METHODS

The guinea pigs were sacrificed for their lungs. The lungs were cut and trimmed to obtain the bronchial rings. These rings were suspended (Fig. 2) under a tension of 2 g in organ bath that contained Krebs-bicarbonate solution, aerated with oxygen. The study was done in the presence or absence of MAPKK inhibitor treatments, with dimethyl sulfoxide as negative control and indomethacin as positive control. The contractile responses were monitored and the data was analysed by using one-way analysis of variance (ANOVA) followed by Tukey test. The critical level for significance was set at P <0.05.

RESULTS

Based on our previous studies (Tsang et al., 1998), PD 098059 markedly potentiated bronchial ring contraction induced by histamine. To determine whether the potentiation effect of PD 098059 on agonist-induced bronchial smooth muscle is dose-dependent or not, we evaluate the effects of increasing concentrations of PD 098059 on histamine- or LTD4-induced bronchial contraction. PD 098059 (10 µM-50 µM) potentiated both histamine- and LTD4-induced bronchial contraction, but did not show dose-dependent effect (Fig. 3A and Fig. 3B). To determine whether U0126 might have the same effects as PD 098059 on agonist-induced bronchial smooth muscle contraction, we evaluate the effects of 30 µM U0126 on histamine- or LTD4-induced bronchial contraction. U0126 (30 µM) significantly (P<0.05) potentiated bronchial ring contraction induced by LTD4 (Fig. 4B), as well as that by histamine (Fig. 4A).
Figure 3. Effects of PD 098059 on (A) 30 µM histamine-, or (B) 0.1 µM LTD₄-induced bronchial contraction. A DMSO control was carried out in parallel with each PD098059 concentration. Each point represents the mean ± S.E.M. of three to six experiments. * Significant difference from DMSO control, P<0.05. DMSO was used in parallel with PD 098059 as negative control.

Figure 4. Effects of 30 µM U0126 on (A) 30 µM histamine-, or (B) 0.1 µM LTD₄-induced bronchial contraction. A DMSO control was carried out in parallel with 30µM U0126. Each point represents the mean ± S.E.M. of three to five experiments. * Significant difference from DMSO control, P<0.05. DMSO and Indomethacin (C & D) were used in parallel with U0126 as negative and positive controls, respectively.
DISCUSSION

Inhibition of MAPKK by PD 098059 prevented the release of relaxant prostanoids such as PGE₂ from human bronchial epithelial cells by inhibiting the activity of ERK (Abela and Daniel, 1994). It is because inactivated ERK cannot activate PLA₂. Therefore COX, which is downstream of PLA₂, as well as the precursor of PGE₂, is inhibited, thus no PGE₂ is produced (Fig. 5). Meanwhile U0126 inhibits IL-1β-dependent PGE₂ release primarily by inhibiting a step (or steps) upstream of COX and this explains our results in Fig. 4. It has been shown that PGE₂ can inhibit early asthmatic response via downregulation of PGs, especially PGD₂, which causes acute early bronchoconstriction in allergic asthma; and PGE₂ also inhibits other mediator release from the mast cell. Thus we speculate that PD 098059 and U0126, by inhibiting MAPKK, attenuated the downstream MAPK activity and the release of PGE₂, leading to enhanced histamine- and LTD₄-induced bronchial contraction observed in the present study. It has been reported that PD 098059 and U0126 make good anti-inflammatory drugs, as well as tumour suppression drugs. There might be a small subgroup of people from these patients who happened to have asthma. If this subgroup takes MAPKK inhibitors as anti-tumour drugs, their anaphylactic bronchoconstriction will be aggravated or may even be fatal.

REFERENCES