Anti-mitogenic effects of bitter taste receptor (BTR) agonists on human airway smooth muscle cells

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Abstract

Rationale: Obstructive diseases of airways such as asthma and COPD are characterized by airway remodeling. Clinical studies and animal models have demonstrated that ASM mass is increased in asthma, and ASM thickness is correlated with severity of the disease. Current asthma medications control inflammation and reverse airway obstruction effectively, yet have very limited effects in deterring airway remodeling. Recently we identified the expression of BTRs on human ASM cells. Activation with known BTR agonists resulted in elevation of intracellular calcium, myosin light chain phosphorylation and ASM relaxation. A novel mechanism of normal and allergic sensitization- and challenge- induced smooth muscle tone is suggested in a robust bronchodilatation. Another recent study demonstrated that BTR expression, signaling and bronchodilatory effects are preserved during human asthma. These studies suggest that BTRs can be used in new therapeutic targets in the clinical management of obstructive lung diseases. The current study aimed at determining the effect of BTR agonists on ASM growth.

Methods: Primary human ASM cells maintained in culture were pretreated with different concentrations of BTR agonists, chloroquine and quinine, or PDGF in presence or absence of hyperplasia. BTR agonists did not induce apoptosis or cell death in human ASM cells. Growth inhibitory effects of BTR agonists in ASM cells were not dependent on protein kinase A (PKA) as demonstrated for other Gi coupled G protein coupled receptor agonists (e.g. β2-agonists and PGE2). Western blot analyses of key mitogenic signaling demonstrated that BTR agonists inhibit mitogen-induced activation of p42/p44, p38 and Akt-agonists and PGE2). Western blot analyses of key mitogenic signaling demonstrated that BTR agonists inhibit mitogen-induced activation of p42/p44, p38 and Akt pathways.

Conclusion: Collectively, these data suggest that BTR agonists inhibit ASM cell growth by inhibiting key mitogenic signaling pathways in ASM via PKA-independent mechanism, suggesting a novel and unexploited mode of inhibiting ASM growth. Future studies are needed to establish in vivo effectiveness of BTR agonists on airway remodeling.

Experimental Findings

Figure 1. Effects of BTR agonists on ASM growth monitored by CyQuant assay. Human ASM cells were pretreated with different concentrations of chloroquine (Chlo), quinine (Quin) or vehicle (Veh) for 15 min and stimulated with FBS (A) or PDGF (B) for 72 h. DNA content was assessed by flow cytometry using CyQuant dye. No significant differences in the size of the cells under different treatment conditions.

Figure 2. BTR agonists inhibit PDGF induced ASM hyperplasia. A. Human ASM cells were pretreated with different concentrations of chloroquine (Chlo), quinine (Quin), or vehicle (Veh) for 15 min and then stimulated with PDGF for 72 h. DNA content was assessed by flow cytometry using CyQuant dye. Note decrease in the number of cells under different treatment conditions.

Figure 3. BTR agonists affect ASM cell cytotoxicity and apoptosis. Human ASM cells were pretreated with different concentrations of chloroquine (Chlo), quinine (Quin), or vehicle (Veh) for 15 min and then stimulated with FBS or PDGF for 72 h. Cell cytotoxicity was determined by ApoTox Green (A) or CyQuant dye (B) and apoptotic effects was determined by caspase-3 assay (C).

Figure 4. Role of PKA and PKC in BTR-induced anti-mitogenic effects on ASM. Human ASM cells were pretreated with PKA-agonists PKA-GF or PKA-GF+Chlo and then stimulated with FBS or PDGF. Concentration response curves of PKA-GF alone were treated with PKA-agonist, PKA-GF+Chlo and PDGF and human ASM cells pretreated with PKA-GF alone or PKA-GF+Chlo and PDGF were subjected to immunoblot analysis for phospho-p42/p44, p38, Akt and PKC. Note significant inhibition of growth factor (PDGF)-induced mitogenic signaling by BTR agonists.

Figure 5. Immunoblot analysis of effects of BTR agonists on mitogenic signaling in ASM. Human ASM cells were pretreated with different concentrations of Chlo or Quin and stimulated with PDGF, and lysates were harvested and subjected to immunoblot analysis for phospho-p42/p44 (A), p38 (B), Akt (C), PKA (D) and PKC (E). Western blot analyses of key mitogenic signaling demonstrated that BTR agonists inhibit mitogen-induced activation of p42/p44, p38 and Akt pathways.

Figure 6. Role of PKA and PKC in BTR-induced anti-mitogenic effects on ASM. Human ASM cells were pretreated with PKA-agonists PKA-GF or PKA-GF+Chlo alone or treated with PKA-agonists, PKA-GF+Chlo and PDGF and then subjected to Western blot analysis for phospho-p42/p44 (A), p38 (B), Akt (C), PKA (D) and PKC (E).

Summary and Conclusion

- BTR agonists, chloroquine, quinine and saccharine inhibit growth in normal and asthma ASM cells in a dose-dependent manner.
- Anti-mitogenic effect of BTR agonists is not due to cytotoxicity or induction of apoptosis in ASM.
- BTR agonists inhibit cell number (hyperplasia) and not hypertrophy.
- Pretreatment of ASM cells with BTR agonists inhibits growth factor-induced cell cycle progression.
- Growth inhibitory effect of BTR agonists does not involve activation of protein kinases such as PKA or PKC.
- BTR agonists inhibit mitogenic signaling (p42/p44, p38, Akt and p70S6K) that regulates growth factor-induced ASM cell proliferation.
- Future studies will determine detailed intracellular signaling and in vivo effectiveness of BTR agonists in reversing ASM remodeling using animal model of asthma.

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