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, membrane hyperpolarization and ASM relaxation. Aerosol challenge in normal and allergen sensitized- and challenged- mice resulted in a robust bronchodilation. Another recent study demonstrated that BTR expression, signaling and bronchodilatory effects are preserved during human asthma. These studies suggest that BTRs can be used as 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 vehicle, then stimulated with ASM mitogens fetal bovine serum (FBS), platelet-derived growth factor (PDGF) or epithelial growth factor (EGF). Regulation of ASM growth was subsequently assessed by cell counts, CyQuant assay and ^{3}H-thymidine-incorporation assays. Parallel studies assessed the effects of BTR agonists on key mitogenic signaling pathways in the ASM by Western blotting.

Results: In CyQuant assays, chloroquine and quinine significantly inhibited growth of normal and astmatic human ASM cells induced by each mitogen in a concentration-dependent manner. BTR agonists also inhibited increases in ASM cell number suggesting their anti-mitogenic effect is mediated via inhibition of hyperplasia. BTR agonists did not induce apoptosis or cell death in human ASM. Growth inhibitory effects of BTR agonists in ASM cells were not dependent on protein kinase A (PKA) as demonstrated for other Gs coupled G protein coupled receptor agonists (e.g. β-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

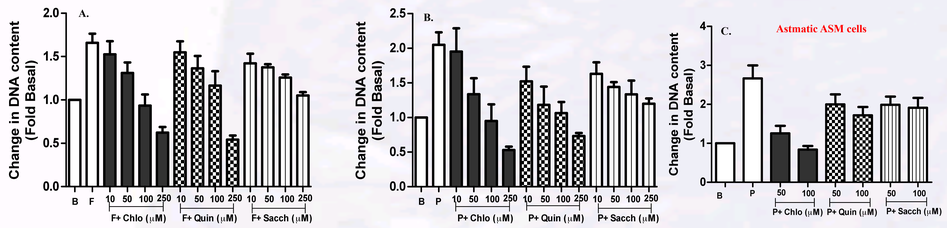


Figure 1. Effects on BTR agonists on ASM growth assessed by CyQuant assay. Human ASM cells were pretreated with different concentrations of chloroquine (Chlo), quinine (Quin) or saccharine (Sacch) for 15 min and stimulated with FBS (A) or PDGF (B) for 72 h. Cell number was determined by CyQuant assay and data presented as fold change in fluorescence from baseline. N=5. B-basal, F-FBS, P-PDGF. C. Studies described in A and B were repeated using "asthmatic" human ASM cells.

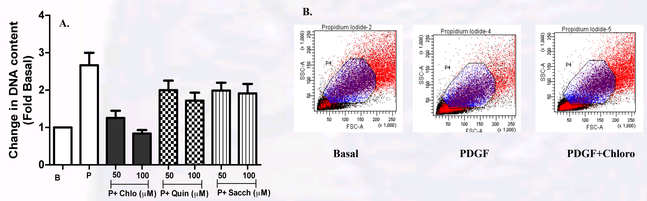


Figure 2. BTR agonists inhibit PDGF-induced ASM hyperplasia. A. Human ASM cells were pretreated with 50 or100 μM chloroquine, quinine or saccharine and PDGF-induced hyperplasia was determined by cell count. B. Flow cytometric analysis (forward scatter) of ASM cells using propidium iodide (PI) staining did not reveal any differences in the size of the cells under different treatment conditions.

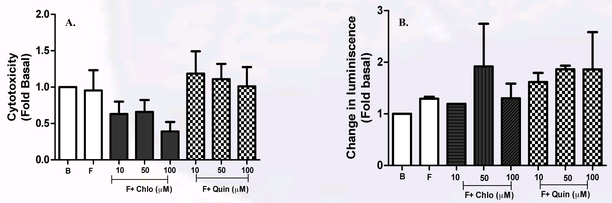


Figure 3. BTR agonist effect on ASM cell cytotoxicity and apoptosis. Human ASM cells were pretreated with different concentrations of chloroquine (Chlo) or quinine (Quin) and treated with FBS (F) for 72 h. Cytotoxicity (A) and Apoptosis (B) were determined luminometrically using ApoTox Glo Triplex assay. There was no change in the luminescence in ASM cells upon treatment with BTR agonists suggesting that BTR agonists do not induce cytotoxicity or apoptosis in human ASM cells. n=2 (B- Basal, F-FBS).

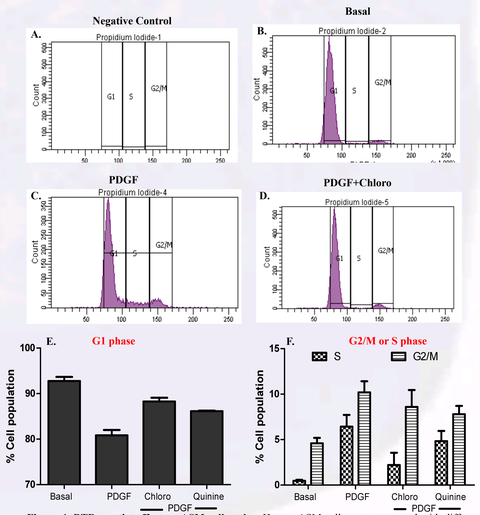
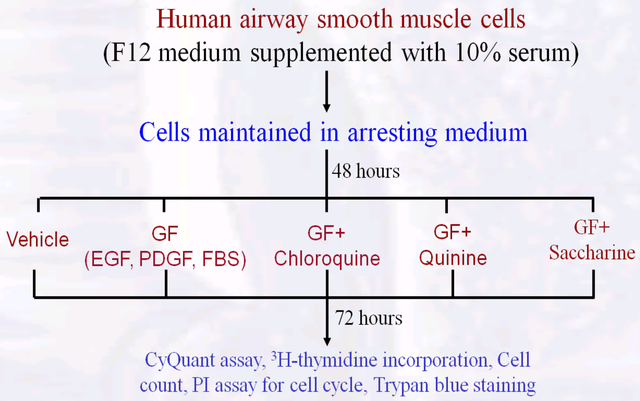
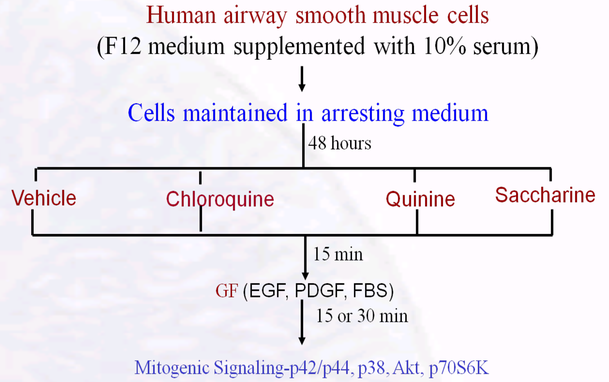


Figure 4. BTR agonist effect on ASM cell cycle. Human ASM cells were pretreated with different concentrations of chloroquine (Chlo) or quinine (Quin) and treated with PDGF for 72 h. Cell cycle was measured by flow cytometry using propidium iodide staining. (A-D) representative traces of PI staining in human ASM cells, (E and F) percentage of cells in different phases of the cell cycle. Note decrease in proportion of cells in G1 phase upon treatment with PDGF and reversal by BTR agonists. n=4 .

Experimental Design





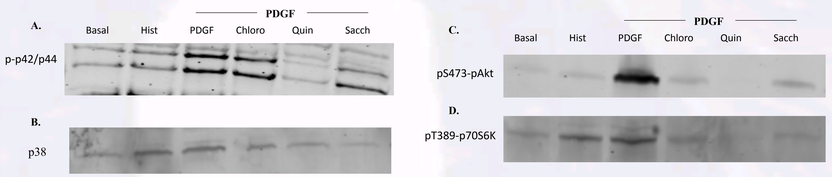


Figure 5. Immunoblot analysis of effects of BTR agonists on mitogenic signaling in ASM. Human ASM cells were pretreated with chloroquine (chloro), quinine (quin) or saccharine (Sacch) and stimulated with PDGF, and lysates were harvested and subjected to immunoblot analysis for phospho-p42/44 (A), p38 (B), pAkt (C) or p70S6K (D). Note significant inhibition of growth factor (PDGF)-induced mitogenic signaling by BTR agonists.

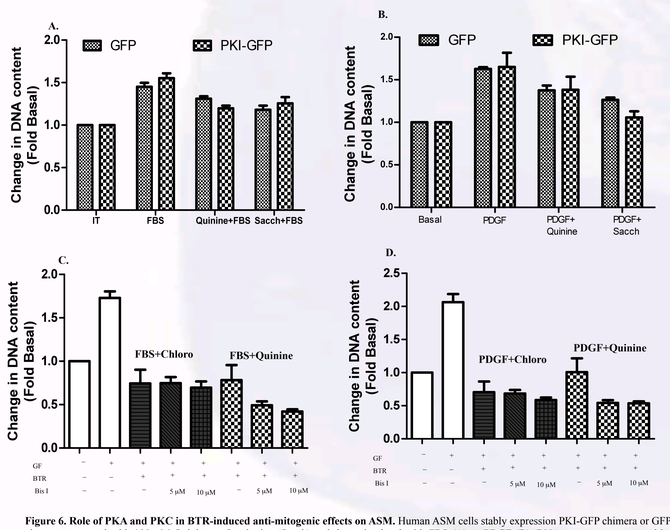


Figure 6. Role of PKA and PKC in BTR-induced anti-mitogenic effects on ASM. Human ASM cells stably expression PKI-GFP chimera or GFP alone were treated with 100 μM Quinine or Saccharine (Sacch) and then stimulated with FBS (A) or PDGF (B). DNA content was assessed by CyQuant assay. n=3. Human ASM cells were pretreated with PKC inhibitor Bis I (5 or 10 μM) followed by treatment with Chloroquine or Quinine, and then stimulated with FBS (C) or PDGF (D). DNA content was determined by CyQuant assay after 72 hours. Antimitogenic effect of BTR agonists was not attenuated by inhibiting PKA or PKC activity in ASM cells.

Summary and Conclusion

- ❖ BTR agonists, choloquine, quinine and saccharine inhibit growth in normal and asthmatic ASM cells in a dose-dependent manner.
- ❖ Antimitogenic 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 with BTR agonists inhibit 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.

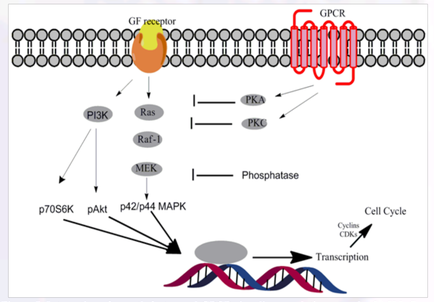


Figure 7. Interaction of growth factor and GPCR signaling regulating growth of ASM. Growth factors such as EGF and PDGF induce ASM growth predominantly via activation of p42/p44 MAP kinase, pAkt, and p70S6K. GPCR agonists via activation of PKA or PKC inhibit mitogen- induced ASM growth.

Supported by AAF