Online Supplement

Methods

Animal studies

In order to determine the thermal profile of BT on the airway wall, we used a white-Landrace piglet which has a similar airway anatomy to that of the adult human lung[1]. The animal was treated in compliance with the Canadian Council for Animal Care and the protocol received approval by the University of Calgary Animal Care and Use Committee (AC14-0135). The animal was euthanized with sodium pentobarbital and the lungs immediately excised *en bloc*. A thermal camera (FLIR SC660, Wilsonville, OR) was utilized to characterize and measure the heat profile produced by the BT device in the excised porcine airways n=3 airways. This camera has a sensitivity of 0.02^{o}C and detects infrared radiation wavelengths between 7.5-13 µm. The BT catheter was inserted into the major airways of the excised lung and multiple 'firings' performed, with each captured by the thermal camera. The thermal camera was calibrated according to manufacturer's instructions and, used in 'camera mode' with 180 images captured in intervals of 1.0ms for a total of 18s bracketing the 10s heating time of the bronchial thermoplasty device. The captured frames were imported and analyzed using the ThermaCAM researcher pro 2.9 software. Images were compressed and exported as a video (Audio Video Interleaved, AVI file).

Cell Culture studies

Primary human bronchial epithelial (HBE) cells and ASM cells were obtained from non-transplanted normal human bronchi from 3 healthy lung donors (13-63y) as described previously[2]. HBE cells were seeded onto 4-chamber slides ($1.0x10^5$ cells/well) in bronchial epithelial cell growth medium (BEGM) containing 5% FBS (37^{o}C, 5% humidity). The media was changed at 72h and the cells grown to 90% confluence at 37^{o}C. ASM cells were seeded onto 4-chamber slides chamber slides ($5x10^4$ cells/well) in Dulbeccco's Modified Eagle Media (DMEM) containing 10% FBS and 100U penicillin, 100U streptomycin, 250ng/ml amphotericin B (PSF) and grown to 90% confluence at 37^{o}C. The cells were then treated with BEGM (HBE cells) or DMEM (ASM cells) heated to 37^{o}C (control), 65^{o}C or 85^{o}C for 10s. These temperatures were chosen based on the results of thermal mapping in the piglet bronchiole studies described above. The thermal profile in each chamber was captured by the thermal camera (FLIR SC660). The thermal camera was used in 'camera mode' with 180 images captured in intervals of 1.0ms. The captured frames were imported and analyzed using the ThermaCAM researcher pro 2.9 software suite. Images were compressed and exported as a video (AVI file). Post-heat treatment, BEGM or DMEM was replaced with that heated to 37^{o}C and the cells were cultured for 24hrs. The cells

were fixed in acetone-ethanol and stained with H&E; the ASM cells were stained with αSMA in addition.

The viability of ASM cells following hyperthermic treatment was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as per manufacturers instruction and assessed with a spectrophotometer at 570 nm (Bio-Rad Benchmark microplate reader #170-6850).

Study participants

Participants (18-65y), diagnosed with asthma and prescribed regular maintenance inhaled corticosteroid (ICS > 1,000mg/d beclomethasone or equivalent) and long acting β-agonist (LABA > 100ug/d salmeterol or equivalent) were recruited from the Foothills Hospital Medical Centre Pulmonology outpatient clinic (Table 1). Exclusion criteria included FEV_1 <60% of predicted value, unstable asthma and/or instability of medication dose, clinically unproved asthma as defined by FEV_1, FVC and methacholine provocation, inability to commit to follow up, physician recommendation of exclusion based on decline in lung function from initial visit. The University of Calgary Conjoint Health Research Ethics Board approved the protocol (REB14-1100). All participants provided written informed consent prior to their participation in the study.

Study protocol

Participants were assessed during five clinic visits over a 13-month period (Figure 4 main text). The pretreatment questionnaire consisted of documentation regarding patient demographics, baseline pre-bronchodilator FEV1, inhaled corticosteroid dose, long-acting β-agonist dose, other asthma medications, Asthma Control Questionnaire (ACQ) and, Asthma Quality of Life Questionnaire (AQLQ); data are summarized in Table 1 main text.

Each participant underwent 4 bronchoscopies within the 13-month study. Standard BT (Alair, Boston Scientific, Natick, MA,) spanned three bronchoscopic procedures, each separated by three weeks[3, 4] (right lower lobe (0wks), left lower lobe (3wks), bilateral upper lobes (6wks) Figure 1). Endobronchial biopsies (which included one cryobiopsy and two forceps biopsies) were taken from a subsegment of the right lower lobe prior to BT; the same subsegment was sampled at 6-weeks and 12-months after the first procedure. In the event of a respiratory exacerbation prior to BT, bronchoscopy was delayed for 4 weeks. Clinical follow-up occurred in the post-treatment period at 1, 3, 6, 9, and 12 months (Figure 4 main text).

Histology

Biopsy specimens were checked for adequate size, as recommended previously[5]. If a biopsy did not consist of sufficient submucosal area it was discarded. Biopsies were fixed in 10% formalin for 24hrs before paraffin embedding. Sections were cut (4μm), deparaffinized and stained with: H&E, Picrosirius Red (PSR [6, 7]) or Periodic Acid Schiff's reagent (PAS). All slides (except H&E) were counter-stained with hematoxylin.

Antigen retrieval was conducted with citric acid (pH 6.0, 20min, ~100°C; for CD31 and s100). Endogenous peroxidase (3.0%) was blocked (20min) before Avidin and Biotin block (Vector Laboratories; #SP-2001, 30min) in 2% normal goat serum (Vector Laboratories; #S1000). Primary antibodies (αSMA- 1:150; Invitrogen Mouse anti-human SMA #MS-113-B1; CD31- 1:40; Dako mouse anti-human CD31 #M0823; S100- 1:200; Invitrogen rabbit anti-human #180046; diluted in Dako Antibody Diluent #S0809) supplemented with 2% normal goat serum was applied at room temperature (αSMA and S100- 90min; CD31- 24h). Secondary antibodies (Goat anti-mouse 1:200, Vector Laboratories, #BA9200 or Goat anti-rabbit 1:200, Vector Laboratories; #BA1000) supplemented with 2% normal goat serum were applied for 45 minutes. Peroxidase labeled Avidin Biotin complex (Vector Laboratories; # PK4000) was applied for 30 minutes. Sections were washed with ddH_2O before DAB chromogen (Sigma-Fast Catalogue #D4168, 5min) and counter-stained with hematoxylin.

Morphometric analysis

Stereological analysis of sections stained with H&E, αSMA, and CD31 were assessed with Stereo Investigator (MBF Bioscience, Williston, VT) in conjunction with a stereomicroscope (Axio Imager.2, Zeiss Toronto, ON) to measure total submucosal area, area/volume fraction of ASM and glands, sub-basement membrane, and vessel cross-sectional area and vessel density.

Goblet cells, identified with PAS stain, were manually counted in intact, well-orientated lengths of epithelium, and normalized to the sub-basement membrane length. Nerves were identified by their distinctive morphology and positive staining with S100 and were expressed per submucosal area. Slides stained with picrosirius red to evaluate collagen were photographed under birefringent conditions, visualized using the Olympus BX51 microscope mounted with an Olympus DP80 camera using cellSens software (Olympus, Richmond Hill, ON) and the total birefringent area was normalized to submucosal area. Red and green birefringent collagen were assessed as a percent of total PSR stained area [6, 8]. In all cases, the investigator was blinded to which samples were examined. Analysed biopsy area was not significantly different between timepoints (Supplemental Figure 1a). Biopsy area was measured on two separate days in random order and a low coefficient of variation (<3±1.2) confirmed good repeatability.

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