Airway remodelling in smokers with or without chronic obstructive pulmonary disease (COPD) and the effects of inhaled corticosteroids on remodelling in COPD

By

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Declaration of Originality

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The research associated with this thesis abides by the international and Australian codes on human and animal experimentation, the guidelines by the Australian Government’s Office of the Gene Technology Regulator and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University.

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**Abstract**

**Introduction:** Smoking-related COPD is a worldwide health problem. Airway remodelling is defined as structural changes occurring in chronic inflammatory diseases of the airways. Our knowledge about airway remodelling in COPD is very limited. My preliminary observational study of bronchial biopsies (BB) from COPD subjects revealed reticular basement membrane (Rbm) fragmentation and vascular changes. I hypothesised that these changes are specific for COPD and are related to the angiogenic activity of vascular endothelial growth factor (VEGF) and transforming growth factor-β (TGF-β). I also aimed to study the effects of inhaled corticosteroids (ICS) on these airway changes.

**Methods:** A cross-sectional study compared BB from current smokers with COPD (S-COPD), ex-smokers with COPD (ES-COPD), current smokers with normal lung function (S-N) and healthy nonsmoking (H-N) subjects. BB were stained with anti-Collagen IV, anti-VEGF and anti-TGF-β antibodies. Rbm fragmentation and vessels in the Rbm and lamina propria (LP) were measured. Anti-Factor VIII antibody was compared with anti-Collagen IV antibody in detecting vessels.

Then a double-blind, randomized and placebo controlled study assessed the effects of ICS on airway remodelling, VEGF and TGF-β in COPD.

**Results:** Airway remodelling changes were also detectable in S-N. The Rbm was fragmented. The length of splits was significantly greater in both COPD groups and in S-N than controls (p<0.02). The Rbm was hypervascular and the LP hypovascular in current smokers compared with controls (p<0.05). Vessels stained for VEGF and TGF-β were increased in the Rbm of both COPD groups and S-N (p<0.05). Factor VIII
antibody confirmed my finding of hypovascularity of the LP in S-COPD. ICS reversed Rbm splitting but did not have any effect on vessel remodelling and angiogenic activities.

**Discussion:** My studies revealed novel aspects of Rbm and vascular remodelling in BB from COPD subjects and S-N and for the first time showed that ICS are effective on Rbm changes in COPD. Rbm fragmentation, we think, is probably a consequence of the effects of proteolytic enzymes on the Rbm due to activation of epithelial-mesenchymal transition (EMT) by smoking. This is under more investigation in our group. My study could not explain the mechanisms to vessel changes in current smokers. Further studies to examine the role of other angiogenic/antiangiogenic factors are now needed. Absence of vascular changes in ES-COPD subjects may imply that vascular remodelling is reversible with smoking cessation, but to test this we need a longitudinal smoking cessation study.
Publications

Publications:


• Sukhwinder S. Sohal, David Reid, Amir Soltani, Chris Ward, Steven Weston, H. Konrad Muller, Richard Wood-Baker, E. Haydn Walters “Reticular basement membrane fragmentation and potential epithelial mesenchymal transition is exaggerated in the airways of smokers with chronic obstructive pulmonary disease.” Accepted for publication in Respirology, August 2010, 15 (6), in press. [Original article]

   http://www.sciencedirect.com/science/journal/01637258

• Walters EH, Soltani A, Reid DW, Ward C. “Vascular remodeling in asthma.” Current Opinion in Allergy and Clinical Immunology, 2008; 8:39-43. [Review article]
   http://journals.lww.com/co-allergy/pages/default.aspx
Conference Presentations and Abstracts

Abstracts:


- **Soltani**, D. Reid, S.S. Sohal, H.K. Muller, S. Weston, R. Wood-Baker, E.H. Walters. “Vascular and basement membrane remodeling in smokers and COPD.” *European Respiratory Journal* 2009; 34: supplement 53, 48s. [Abstract]. This abstract was presented as an E-communication in the
S.S. Sohal, D. Reid, A. Soltani, C. Ward, S. Weston, H.K. Muller, R. Wood-Baker, E.H. Walters. “Smoking has potential to initiate basement membrane disruption and epithelial mesenchymal transition in COPD.”


Abstract listed below is not directly related to the content of my thesis:

• **Soltani A**, Reid D, Almond I, Walters EH, Wood-Baker R. “Survey of comorbidities in acute exacerbations of chronic obstructive pulmonary disease.” **Respirology** 2009; 14: Supplement 1, A53. [Abstract]. This abstract was presented as a poster in the TSANZ Annual Scientific Meeting in Darwin, April 2009.

  [http://www3.interscience.wiley.com/cgi-bin/fulltext/122257085/PDFSTART](http://www3.interscience.wiley.com/cgi-bin/fulltext/122257085/PDFSTART)
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Abbreviations and symbols

Abbreviations
AECOPD = Acute exacerbations of COPD
AHR = Airway hyper-responsiveness, same as BHR
AM = Alveolar macrophage
ATS = American Thoracic Society
BAL = Broncho-alveolar lavage
BALF = Broncho-alveolar lavage fluid
BB = Per-bronchoscopic bronchial biopsies
BDR = Bronchodilator responsiveness
BM = Basement membrane
bFGF = Basic fibroblast growth factor
BHR = Bronchial hyper-responsiveness
CD (#) (like CD31 etc.) = Are surface antigens that are detectable by using different antibodies and are used to address different kinds of cells in the hematopoietic and tissue mononuclear-macrophage cellular system.
Cm = centimeter
CoR = Coefficient of repeatability
CT-scan = Computerized tomography scan
Dmin = The dose of methacholine that provokes 20% decrease in FEV1. It shows the presence and the severity of BHR.
ECM = Extracellular matrix
ELISA = Enzyme linked immunosorbent assay
EMT = Epithelial-mesenchymal transition
ERS = European Respiratory Society
ES-COPD = Exsmoker COPD
FEV1 = Forced expiratory volume in first second
FER = Forced expiratory ratio = FEV1/FVC ratio x 100
FOB = Fiberoptic bronchoscope
FP = Fluticasone propionate
FVC = Forced vital capacity
DNA = deoxy ribonucleic acid
GOLD = Global Initiative for Chronic Obstructive Lung Disease
GR = Glucocorticoid receptor
GM-CSF = Granulocyte-macrophage colony stimulating factor
H-N = Healthy and nonsmoker
HRCT = High resolution computerized scan
Hyper- = A prefix that means increase of, e.g. hypervascularity means increased vessels
Hypo- = A prefix that means decrease of, e.g. hypovascularity means decreased vessels
IC = Inspiratory capacity
ICS = Inhaled corticosteroid/inhaled corticosteroids
I-κB = Inhibitor of κB
IL = Interleukin
LABA = Long-acting beta-adrenergic agonist
LoA = Limits of agreement
LP = lamina propria
MAPK = Mitogen-activated protein kinase
mg = milligram
mm = millimeter
MMP = Matrix metalloproteinase
mRNA = Messenger ribonucleic acid
NF-κB = Nuclear factory-kappa B
NO = Nitric oxide
PCR = Polymerase chain reaction
PEF = peak expiratory flow
Percent vascularity = Area of vessels/area of the lamina propria examined
Pi = Protease inhibitor = Alpha1-antitrypsine
PI3K = Phosphoinositide 3 kinases
PMN = Polymorphonuclear leukocyte
Rbm = Reticular basement membrane
RNA = Ribonucleic acid
ROS = Reactive oxygen species
S-COPD = Current smoker COPD
SD = Standard deviation
SE = Standard Error
SFC = Salmeterol + fluticasone propionate
SGRQ = St George’s Respiratory Questionnaire
S-N = Smokers with normal lung function
SNP = Single nucleotide polymorphism
TGF-β = Transforming growth factor-beta
TIMP = Tissue inhibitor of metalloproteinases
Vascular density = Number of vessels/ area of the lamina propria examined
%vascular area = Percent vascular area
VEGF = Vascular endothelial growth factor
VEGFR = Vascular endothelial growth factor receptor
VEGFR-1 = Flt-1, fms-like tyrosine kinase
VEGFR-2 = KDR/FLK-1, Kinase-insert domain receptor/fetal liver kinase
VEGFR-3 = Ftl4, fms-like tyrosine kinase 4

Symbols:
α = Alpha
β = Beta
γ = Gamma
μ = Micro
μm = Micrometer
ν = Nu
κ = Kappa
I = one
II = Two
III = Three
IV = Four
V = Five
VIII = Eight
FOR:
Parisa, Ehsan and Sara:
   - My love, family and life.
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