Aquaporins:

A Channel to Understanding the Pathogenesis of Chronic Rhinosinusitis

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Thesis submission date: 10th December 2014

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Thesis Summary

Aquaporins (AQPs) are cell membrane water transport channels and their discovery has revolutionised the understanding of water movement through tissue and tissue remodelling in the last 20 years. In chronic rhinosinusitis (CRS), there are pathological features suggestive of aberrant sinonasal water transport including altered composition of secretions, mucosal oedema, tissue remodelling and polyp formation. This project was undertaken to investigate a possible link between AQPs and CRS.

Chronic rhinosinusitis (CRS) is a chronic, inflammatory condition of the nose and paranasal sinuses that affects up to 10% of the Australian population. Characterised by inflammation of the sinonasal mucosa, CRS was defined by the European Position Paper on Rhinosinusitis and Nasal Polyps 2012 (EPOS 2012) based on clinical symptoms and investigation findings. Chronic rhinosinusitis (CRS) is further classified phenotypically into CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP). However, AQP expression in normal and CRS sinus tissue remains incompletely understood. This study provides baseline knowledge of sinonasal mucosa AQP expression for the future investigation of AQPs in the pathogenesis of CRS. The hypothesis of this thesis was that AQP expression and location are altered in CRS in comparison to normal sinonasal mucosa.

Methods

Sinonasal tissue was collected during endoscopic sinus surgery or trans-sphenoidal surgery from three patient groups: normal controls, CRSwNP and CRSsNP. The mRNA expression of human AQP0–AQP12b was determined using quantitative real-time PCR. Cellular localisation of AQP1, AQP3, AQP4, AQP5, AQP7 and AQP11 was determined by immunohistochemistry.

Results

The mRNA of AQP0–AQP11 was identified in all samples; however, AQP12b mRNA was not detected. Statistically significant differences in the mRNA expression levels of AQP4 and AQP11 were identified between normal and CRSwNP patients ($p<0.05$). Differences in the cellular localisation of AQPs were observed in both CRSsNP and CRSwNP patients vs. normal controls. More intense localisation to the cell cytoplasm was observed for AQP5 in glandular epithelium

(CRSwNP; $p<0.05$) and surface epithelium (CRSsNP; $p<0.05$), and AQP4 in glandular epithelium (CRSsNP; $p<0.05$).

Conclusion

This study characterises normal human sinonasal AQP mRNA expression and protein localisation. The findings correlate well with the few published studies in this area, and extend the knowledge of AQP expression in human sinonasal tissue by providing normal baseline AQP expression profiles for future reference. Increased intracellular localisation of AQP4 and AQP5 was identified in both phenotypes of CRS, raising interesting questions regarding the significance of these findings in CRS aetiology. Future work will focus on the implication of intracellular AQPs on water flow and/or tissue remodelling in CRS.

Declaration

I certify that this thesis does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any university; and that, to the best of my knowledge and belief, it does not contain any material previously published or written by another person except where due reference is made in the text.

Dr Claire Frauenfelder

Acknowledgements

Completion of this project has been a team effort and I gratefully acknowledge the assistance of my supervisors and mentors, Professor A.S. Carney, Dr Charmaine Woods, Dr Damian Hussey and Dr Eng Ooi.

Ethics and tissue selection

The ethics application was completed as part of a larger project commenced prior to my time in the department by Dr Charmaine Woods in conjunction with other members of Flinders ENT. Some samples used in this project were obtained from the Flinders ENT tissue bank and RNA had already been extracted. During the course of the project, I actively recruited patients to contribute tissue samples to this study, as well as the tissue bank. I personally identified suitable patients for inclusion and meticulously checked all clinical records (from both public and private practice case records), imaging and pathology to ensure that each participant was adherent to the inclusion and exclusion criteria.

PCR component of study

After training by Dr Woods, Dr Hussey and research assistants Tingting Wang and Alfiya Ansar in the Flinders University Upper Gastrointestinal (GI) Laboratory, I extracted RNA from newly acquired samples. I then performed all spectrophotometry, RNAase treatment, cDNA synthesis, real-time quantitative polymerase chain reaction (PCR) and verification of PCR products. An electronic workbook for recording experimental data in line with local laboratory protocols was kindly modified for this project with the assistance of Dr George Mayne. The data normalisation and analysis were performed with input from Dr Hussey and Dr Woods, with some training from Tingting Wang.

Immunohistochemistry component of study

The formalin-stored samples were processed and paraffin blocked at the time of collection by SA Pathology at a fee to the department. Immunohistochemistry (IHC) slides were prepared from the paraffin blocks by research assistant Kim Griggs from the Department of Anatomical Pathology for a fee. Anti-AQP antibodies 1, 3, 4 and 5 had previously been optimised by Ms Griggs during departmental investigations, and she supervised my acquisition of skills in tissue and slide preparation, IHC

labelling and antibody optimisation for the remaining AQPs. Microscopy training was provided by Yvette DeGraff as part of my orientation to the Flinders Microscopy facilities. The scoring system and skills in reading AQP-labelled slides were developed in conjunction with Associate Professor Sonja Klebe. Further assistance and training in slide reading and scoring were provided by Dr Woods (Flinders ENT), Kim Griggs and Dr David Astill (Department of Anatomical Pathology, Flinders Medical Centre). Statistical advice regarding the use of Likert scale data was sought during a consultation with Associate Professor Richard Woodman, School of Medicine, Flinders University.

Thesis proof-reading

The thesis was proof read by Valerie Williams (Professional Writing Services).

Additional contributors

To my friends, siblings and colleagues: thank you for your patience and support.
To my mother, Judy Frauenfelder; we have walked a hard road together: thank you.

Finally, completion of this thesis would not have been possible without the dedicated support of my husband, Dr Eamon Raith. You have been there for every up and the myriad of downs. Your encouragement, patience, love of academia and wise advice have kept me going and I am eternally grateful.

Publication arising from this thesis

Aquaporin expression profiles in normal sinonasal mucosa and chronic rhinosinusitis.

Frauenfelder C, Woods C, Hussey D, Ooi E, Klebe S, Carney AS.

International Forum of Allergy and Rhinology. November, 2014;4:901-908

Presentations arising from this thesis

2013 **Aquaporins: a channel to understanding chronic rhinosinusitis?**
American Rhinologic Society Annual Meeting, Vancouver, Canada

2013 **Altered sinonasal aquaporin mRNA expression in chronic rhinosinusitis – a new focus**
The Australian Society for Medical Research:
2013 South Australian Scientific Meeting

2012 **Water transport through sinonasal mucosa: a role for aquaporins in chronic rhinosinusitis?**
The Australian Society for Medical Research:
2012 South Australian Scientific Meeting

List of abbreviations

18S rRNA: 18S ribosomal ribonucleic acid
AQP(s): Aquaporin(s)
ASL: Airway surface liquid
ATP: Adenosine triphosphate
BBB: Blood-brain barrier
cDNA: Complementary DNA
CFTR: Cystic fibrosis transmembrane conductance regulator
cAMP: Cyclic adenosine monophosphate
cGMP: Cyclic guanosine monophosphate
CRS: Chronic rhinosinusitis
CRSsNP: Chronic rhinosinusitis without (sans) nasal polyps
CRSwNP: Chronic rhinosinusitis with nasal polyps
CT: Computed tomography
DNA: Deoxyribonucleic acid
ECM: Extracellular matrix
ER: Endoplasmic reticulum
FAK–MAPK: Focal adhesion kinase–mitogen-activated protein kinase pathway
FESS: Functional endoscopic sinus surgery
gDNA: Genomic deoxyribonucleic acid
H&E: Haematoxylin and eosin
HPRT: hypoxanthine phosphoribosyl transferase
IHC: Immunohistochemical
MIP: Major intrinsic protein of cell membrane
MMP: Matrix metalloproteinase
mRNA: Messenger
NPA motif: Asparagine-proline-alanine motif
PCL: Periciliary layer
PCR: Polymerase chain reaction
P_f: Tissue water permeability
PKA: Protein kinase
PKC: Protein kinase C
PI3K/Akt: Phosphoinositide 3-kinase/protein kinase B
qRT-PCR: Quantitative real-time polymerase chain reaction
RNA: Ribonucleic acid
RS: Rhinosinusitis
RT: Reverse transcription
SCC: Squamous cell carcinoma
TIMP: Tissue inhibitors of metalloproteinases
UPW: Ultra-pure water