MUC5AC Upregulation by Bile Salts: An In vitro Study

Mahmoud El-Sayed Ali1,2*, Shruti Parikh1, and Jeffrey P Pearson1

1Institute for Cell and Molecular Biosciences, Newcastle University, UK
2Department of Otolaryngology, Mansoura University Hospital, Egypt

Abstract

Introduction: Bile acids, as a constituent of refluxed and aspirated duodenal juice, could alter upper and lower airway major mucins such as MUC5AC in the airway mucosa. Human goblet cell line derived from the human colon carcinoma cells HT29-MTX has been found to express MUC5AC as the main mucin and could be employed to study this hypothesis.

Methods: The cell line HT29-MTX was challenged by various concentrations of a physiologic combination of bile salts. We modified an ELISA technique to measure the expressed MUC5AC within the culture media.

Results: The cultured HT29-MTX cells viability was maintained when challenged with bile salts up to 20 µmol/L. MUC5AC mucin production was upregulated by bile salts in a dose dependant manner within the first 24 hours.

Discussion: Amongst the other constituents of duodenal reflux that reaches the upper and lower airways, bile salts could represent an important element of airway mucin alteration by upregulating MUC5AC production. This effect could be direct due to stimulated mucin release from cell stores or indirect via the release of pro-inflammatory cytokines which stimulate mucin biosynthesis and release.

Conclusion: Bile salts challenge of the HT29-MTX cell line resulted in MUC5AC upregulation and this effect could be extrapolated to the airway goblet cells.

INTRODUCTION

Gastric and duodenal reflux is a common disease and the refluxate entering the upper aerodigestive, then the lower airways could alter the mucosal secretory elements to chemical injury of reflux. Bile salts and trypsin/chymotrypsin are the main components of duodenal reflux. Bile salt aspiration has previously been documented in paediatric and adult populations [1, 2]. As mucus hypersecretion is a key element in airway inflammation and epithelial damage [3, 4], there is a potential of aspirated bile salts to alter mucin expression by the airway mucosa. Previous studies have also shown that bile salts can up-regulate mucin genes e.g. the MUC4 gene in oesophageal cancer [5].

We, therefore, assumed that bile salts could also alter mucin expression in the airways. This study investigates the effect of a mixture composed of various bile salts in proportions similar to those present in human bile on mucin production using a goblet cell model of MUC5AC, one of the major mucins expressed in upper and lower airways.

METHODS

Goblet cell culture (HT29-MTX)

A human goblet cell line derived from human colon carcinoma cells HT29, adapted to increasing concentrations of methotrexate (MTX), was cultured according to a previously described protocol [6]. Cell viability was measured with the CellTiter-Blue® Cell Viability Assay (Promega, USA) in a 96-well plate (Maxisorp, NUNC).

Using 24 well plates, cells were seeded at ~50000/well and challenged with a physiological mixture of bile salts (50% cholic acid, 30% chenodeoxycholic acid, 15% deoxycholic acid and 5% lithocholic acid) [7] at concentrations of 3, 6, 9, 12, 15, 18, 20 µmol/L. We used negative controls containing media free from bile salts and blank controls by omitting the primary antibody. At 24, 48 and 72 hours, 0.2ml media was collected and assayed for MUC5AC using a developed ELISA.

ELISA for the measurement of MUC5AC mucin

Mucin was isolated from the non mucin components in the culture media of the HT29-MTX cells by Caesium Chloride (CsCl) equilibrium density gradient centrifugation [8]. Centrifuged samples were fractionated and assayed for mucin glycoprotein by periodic acid Schiff assay and mucin rich fractions were pooled together and subjected to a 2nd CsCl centrifugation for mucin purification. Baseline mucin production was collected and assayed for MUC5AC using a developed ELISA.
primary antibody at 1:200 (abcam, UK) followed by anti-mouse secondary (peroxidise conjugated) at 1:5000 and casein blocking buffer and antibody diluent.

MUC5AC mucin concentration was measured in duplicate using the locally developed indirect ELISA and absorbance was measured at 405 nm with a plate reader (Bio-Tek EL808).

**Statistical analysis**

We used One-Way ANOVA (Repeated Measures ANOVA, Dunn’s Multiple Comparison post test) and non-parametric unpaired tests (Spearman’s correlation test) with Prism (GraphPad Software Inc., La Jolla, California, USA) with a significant p-value of ≤0.05.

**RESULTS**

The cultured cells remained viable through all the experiment conditions and there was no evidence of cell death at any of the challenge conditions.

Basal MUC5AC mucin secretion in the controls at all time points was 40 µg/ml. MUC5AC production was stimulated by bile salts (Figure) in a concentration dependant manner. Maximal MUC5AC secretion was noted at 20 µmol/L bile salts and the level remained the same at 24 h [50 ± 0.9 µg/ml (mean ± SD)], 48 h [50 ± 0.5 µg/ml (mean ± SD)] and 72 h [50 ± 0.5 µg/ml (mean ± SD)] above the baseline levels (Figure 1).

**DISCUSSION**

This study investigated the effect of bile salts in proportions similar to those in human bile on goblet cell production of MUC5AC. Previous work conducted in our lab [9] has shown that exposure of primary bronchial epithelial cells to bile salt challenge resulted in cell injury and death. Previous studies showed that HT-29 cell line express mainly MUC5AC [10, 11] and as we found that this cell line remained viable to bile salt challenges up to a concentration of 20µmol/L, we think that these cells could be used as a model for airway goblet cells.

The MUC5AC mucin expression by the HT-29 cell line response to bile salt challenge developed and reached its maximum within the first 24 hours and continued after that at nearly the same level. Therefore, for further bile salt stimulation experiments at these concentrations it would be important to analyse the media at multiple time points up to 24 hours to obtain more clear information as regard to the temporal up regulation of MUC5AC production. The increase in MUC5AC production was close to linear up to 20 µmol/L bile salts and the viability of the HT-29 cells was maintained at 100% in those conditions. The concentration of bile salts could therefore be increased above the 20 µmol/L concentration used in this study to find out if higher concentrations would compromise the viability of the HT-29-MTX cells and to find out if and at what bile salt concentration MUC5AC production by the viable HT-29-MTX cells becomes maximal.

MUC5AC up regulation by bile salts could be achieved by a direct effect of bile salts on the mucin expression at the mRNA levels or by the release of MUC5AC from cell stores. It could also be an indirect effect through the release of pro-inflammatory cytokines, such as tumour necrosis factor alpha, Interleukin (IL)-6 and IL-17 [12, 13]. Previous work in our lab has shown that MUC5AC expression was upregulated by IL-8 [14] and bacterial lipopolysaccharide and IL-8 [15]

This study suggests that bile acid aspiration among the other constituents of duodenal reflux could be involved in the airway inflammation and epithelial damage. As bile salt aspiration has previously been reported in both the paediatric and adult populations, bile acid reflux should be considered as a contributor to lung injury in upper and lower airway diseases.

**CONCLUSION**

MUC5AC was upregulated by bile salts and the maximum production of MUC5AC was achieved within the first 24 hours of bile salt challenge.

**REFERENCES**

6. Lesuffleur T, Barbat A, Dussaulx E, Zwiebaum A. Growth Adaptation to Methotrexate of Ht-29 Human Colon-Carcinoma Cells Is Associated With Their Ability to Differentiate into Columnar Absorptive and...


