**Abstract**

Cystic fibrosis is a genetic disorder which results in mutated cystic fibrosis transmembrane conductor regulator protein (CFTR). There are several different mutations; however, they all result in a lack of CFTR activity at the membrane of epithelial cells. Ultimately Cl⁻ secretion is inhibited along with fluid transport across the epithelia. The aim of the research project was to find out whether or not the drug lubiprostone (LUB), when exposed to the basolateral membrane of human bronchial epithelial (HBE) cells, acts upon EP₄ receptors (prostanoid receptors) to activate CFTR in the membrane. LUB is commonly used to treat patients with chronic idiopathic constipation, and irritable bowel disease with constipation; it promotes Cl⁻ secretion in the gut. Previous research shows that LUB does in fact act upon EP₄ receptors on the apical membrane of HBE cells to active CFTR; however, it has not yet been concluded as to which receptors it would act upon if introduced on the basolateral side of the cell. We used a specific EP₄ receptor antagonist (GW627368X) to determine whether or not LUB acts upon these receptors, and we use a CFTRinhib172 to determine whether or not CFTR has been activated in the membrane.

The experiment was performed in an Ussing chamber which allows exposure, of a single side of a population of cells (basolateral), to a compound/drug and to indirectly measure net ion transport. Cells were cultured in flasks, and then seeded onto inserts (permeable artificial membranes) ready for testing. Each day EVOM (a general measure of cell resistance) was measured, and once a high level of resistance was reached, they were ready to be used. When an insert with high resistance was achieved, the insert was set for using in the experiment. The chamber contained high and low chloride solutions on the basolateral and apical sides, respectively. A gas mixture (5% CO₂) was bubbled through the solutions.

Inserts were mounted in the chamber and transepithelial potential (Vte) measured. Each minute 10 µA of current was injected and from the shift in potential the resistance and short circuit current (ISC) determined. After 5 minutes, 1µM LUB was added to the basolateral side of the membrane. Readings were taken each minute until a steady state (ss) was achieved, after which, 10µM CFTRinhib172 was added to the apical membrane, and further readings taken in the same manner until ss was reached. For some inserts 10µM GW627368X (a known EP₄ receptor antagonist) was added to the basolateral side. Once a number of readings were taken, and found to be stable, 1µM LUB was added. As before, readings were taken until ss was achieved, before adding 10µM CFTRinhib172 and taking further readings until ss. Statistical significance was tested using ANOVAS and Student’s t test, and assumed at the 5% level.

LUB produced a positive shift in potential from 3.88 ± 2.22mV to 6.06 ± 2.70mV, before reaching ss at 5.37 ± 2.72mV. We also observed an increase in ISC from 4.44 ± 2.77µA/cm² to 7.83 ± 3.36µA/cm², prior to maintaining ss at 6.36 ± 2.86µA/cm². CFTRinhib172, however,
produced a negative shift from 5.37 ± 2.72mV to 3.13 ± 1.99mV, and ISC showing a similar trend from 7.83 ± 3.36µA/cm$^2$ to 6.36 ± 2.86µA/cm$^2$ (n=23). LUB in the presence of GW627368X resulted in a similar trend, increasing in potential from 2.86 ± 1.68mV to 4.93 ± 2.77mV, and then reaching ss at 4.71 ± 2.65mV. ISC also showed increase from 2.64 ± 1.23µA/cm$^2$ to 5.48 ± 2.55µA/cm$^2$, stabilising at 5.18 ± 2.50µA/cm$^2$. CFTR$\text{inhib}172$ produced a decrease in potential, from 4.71 ± 2.65mV to 2.91 ± 1.79mV and the ISC also decreased from 5.18 ± 2.50µA/cm$^2$ to 2.86 ± 1.31µA/cm$^2$. There was no significant difference in the effect of LUB in the absence or presence of GW627368X.

These data suggest that LUB does not act upon EP$_4$ receptors on the basolateral membrane of HBE cells. Rather an unidentified pathway is activating CFTR in the membrane in response to the basolateral addition of LUB.