Impact of lubiprostone on CFTR function in human bronchial epithelial (HBE) and cystic fibrosis bronchial epithelial (CFBE) cells

The Science

Introduction

The common genetic disorder cystic fibrosis is an autosomal recessive disease with around 60,000 affected people worldwide, occurring in 1 in 2500 births in the UK. It is caused by mutations of the cystic fibrosis (CF) transmembrane conductance regulator (CFTR) gene (Griffiths et al, 1999). CFTR is a Cl⁻ channel essential in Cl⁻ transport in organs such as intestines, lungs, sweat glands, kidneys, pancreas. In CF the main impact is on the lungs (Lakhani et al, 2009), where abnormal CFTR protein disrupts Cl⁻ and water transport, leading to airways clogging, inflammation and lung tissue degeneration. As a result, 70% of patients with CF die due to a lack of oxygen caused by lung damage.

The aim of the project was to investigate the effects of lubiprostone on CFTR function in human bronchial (HBE) and cystic fibrosis bronchial epithelial (CFBE) cells. Previously it has been identified that lubiprostone stimulates Cl⁻ secretion from tracheal submucosal glands (Joo et al, 2009) and human colon carcinoma cells (Ao M. et al, 2010). It can also activate non-CFTR-chloride secretion in CF mice (MacDonald et al., 2008).

Methods

16HBE14o- and CFBE cells were plated onto inserts and were grown so there were no gaps between cells. Inserts were mounted in Ussing chambers with standard Krebs solution at one side and low Cl⁻ Krebs at the other side of the insert. Transepithelial potential (V_{te}) and resistance measurements were taken using Axoscope. Control measurements were recorded for 10 minutes and then lubiprostone added to insert. After 10 minutes CFTR_{inhibitor-172} (which inhibits CFTR) was then added to provide an indication of CFTR function. In test experiments this was repeated in the presence of PKI (which would be expected to prevent activation of CFTR) and also in CFBE cells. Experiments also examined the effect of lubiprostone after addition of CFTR_{inh172}. Statistical significance was tested using analysis of variance (ANOVA) and significance was assumed at the 5% level.
**Results**

In HBE control inserts (n=10) addition of lubiprostone increased current flow, with this increase in current sensitive to CFTR\textsubscript{inh172}, Figure 1A. In CFBE cells (n=4) lubiprostone was without effect, while in the presence of CFTR\textsubscript{inh172} the response to lubiprostone was blunted (n=5). With PKI the response was greater (n=7), Figure 1B. The CFTR\textsubscript{inh172}– sensitive current was significantly reduced in CFBE cells, but was unchanged in inserts treated with PKI, Figure 1C.

![Figure 1A](image)

*Figure 1: The mean data showing short circuit currents (SCC) (\(\mu A/cm^2\)) of control, increase to peak lubiprostone and response to CFTR\textsubscript{inh172} * indicates significant difference to control

**Scientific Discussion**

These data support the hypothesis that lubiprostone activates Cl\(^-\) secretion in airway cells. This activation was mediated by CFTR, as addition of CFTR\textsubscript{inh172} reversed this activation, in CFBE cells there was little response to lubiprostone, and pre-addition of CFTR\textsubscript{inh172} completely attenuated the lubiprostone response. Pre-incubation with PKI of HBE cells had no effect in size response to CFTR\textsubscript{inh172} in comparison to the control. This suggests that protein kinase A (PKA - which is inhibited by PKI) had no role in activation of CFTR. This was unexpected since CFTR is a PKA activated Cl\(^-\) channel and it is believed that this is the principle mechanism for its activation. However it has been shown previously that activating other pathways in the sweat duct can...
stimulate CFTR Cl⁻ conductance (Reddy and Quinton, 2000). This highlights the complex nature of the regulation of CFTR. Overall these data suggest that the main channel activated by lubiprostone is CFTR, and that few if any other Cl⁻ channels are involved. This suggests that lubiprostone will not be a useful drug in the treatment of airway disease in CF patients.

Reflection

What were the most important or surprising aspects of your findings? How did your findings help you to better understand the research context? How does it contribute, support or contradict work by other researchers? What questions still remain? Were there any problems with your proposed methodology? What was the most important learning experience for you?

Throughout the six weeks working on my project I have developed my analytical and problem solving skills. I have gained deeper understanding of the practical skills, sophisticated laboratory techniques used in biomedical science e.g. Ussing chamber technique, cell culture, general laboratory maintenance, the importance of keeping accurate records and statistical analysis of data. Doing the summer research in Dr Robson’s laboratory not only helped me to develop my laboratory skills but also most certainly increased my knowledge base in biomedical research.

References

1. Ao M. et al, (2010), Lubiprostone activates Cl⁻ secretion via cAMP signalling and increases membrane CFTR in the human colon carcinoma cell line, T84, Dig Dis sci (2011) 56: 339-351