Vardenafil corrects chloride transport across F508del-CFTR intestinal mucosa

B. Dhoooghe, S. Noel, C. Bouzin, P. Lebecque, P. Wallenmacq, T. Leal. 1Université Catholique de Louvain, Louvain Center for Toxicology and Applied Pharmacology (LTAP), Brussels, Belgium; 2Université Catholique de Louvain, Pôle de Pharmacologie et Thérapeutique, Brussels, Belgium; 3Cliniques Universitaires St Luc, Pediatric Pulmonology & Cystic Fibrosis, Brussels, Belgium

Objectives: Nasal potential difference measurements have previously shown that vardenafil, a phosphodiesterase type 5 inhibitor, improves CFTR-mediated chloride secretion across the nasal mucosa of mice homozygous for the F508del mutation (CF). This work aimed at studying the potential of vardenafil to rescue CFTR function across the rectal mucosa, representative of the GI tract.

Methods: Distinct rectal potential difference (RPD) profiles were obtained in CF and normal homozygous wild-type mice (WT). Sodium absorption, measured by the response of 10−3 M amiloride (in the presence of 5 × 10−3 M barium to block potassium channels), was much higher in CF (40 ± 4.0 mV) than in WT mice (20.0 ± 1.8 mV; p < 0.001). Chloride secretion recorded in the presence of chloride-free solution and 10−3 M forskolin was twice as low in CF (−4.2 ± 0.5 mV) as in WT mice (−9.4 ± 0.9 mV; p = 0.002). Heterozygous mice showed preserved sodium transport (22 ± 1.9 mV) but reduced chloride secretion (−5.4 ± 1.5 mV). Chloride secretion was restored in CF mice treated with a single intradose of 0.14 mg/kg vardenafil; values reached after treatment (−9.3 ± 1.2 mV) were similar to those obtained in untreated WT mice.

Conclusion: Our findings pointed out the rectal mucosa as an additional target tissue to study in vivo ion transport abnormalities. The RPD test discriminates between CF and non-CF and can be used to investigate the efficacy of therapeutic strategies to rescue CFTR function. As for the airways, vardenafil restores chloride secretion across the GI epithelium. Immunolocalization of CFTR protein in colon tissue preparations are under investigation in KO, CF and WT mice treated or not-treated with vardenafil.

Carbachol and forskolin stimulated bicarbonate transport across human rectal biopsies is dependent on functional CFTR

M.J. Hug, T. von Massenbach, M. Licker, A. Heinemann. 1University Medical Center Freiburg, Pharmacy, Freiburg, Germany; 2University Medical Center Freiburg, Department of Paediatrics and Adolescent Medicine, Freiburg, Germany

The measurement of electrolyte transport through rectal biopsies is a useful ex vivo tool to diagnose Cystic Fibrosis (CF). However, the role of bicarbonate transport by colonic epithelia is only poorly understood. For the present study rectal biopsies were obtained from pediatric CF and non-CF patients and subjected to measurement of transepithelial voltage (Vte) and resistance using a modified perfused Ussing chamber. Stimulation of anion secretion by carbachol (CCH) led to a transient lumen negative deflection of Vte in non CF tissues and a positive deflection in chamber. Stimulation of anion secretion by carbachol (CCH) led to a transient lumen negative deflection of Vte in non CF tissues and a positive deflection in chamber. Stimulation of anion secretion by carbachol (CCH) led to a transient lumen negative deflection of Vte in non CF tissues and a positive deflection in chamber.

Objectives: Nasal potential difference measurements have previously shown that vardenafil, a phosphodiesterase type 5 inhibitor, improves CFTR-mediated chloride secretion across the nasal mucosa of mice homozygous for the F508del mutation (CF). This work aimed at studying the potential of vardenafil to rescue CFTR function across the rectal mucosa, representative of the GI tract.

Methods: Distinct rectal potential difference (RPD) profiles were obtained in CF and normal homozygous wild-type mice (WT). Sodium absorption, measured by the response of 10−3 M amiloride (in the presence of 5 × 10−3 M barium to block potassium channels), was much higher in CF (40 ± 4.0 mV) than in WT mice (20.0 ± 1.8 mV; p < 0.001). Chloride secretion recorded in the presence of chloride-free solution and 10−3 M forskolin was twice as low in CF (−4.2 ± 0.5 mV) as in WT mice (−9.4 ± 0.9 mV; p = 0.002). Heterozygous mice showed preserved sodium transport (22 ± 1.9 mV) but reduced chloride secretion (−5.4 ± 1.5 mV). Chloride secretion was restored in CF mice treated with a single intradose of 0.14 mg/kg vardenafil; values reached after treatment (−9.3 ± 1.2 mV) were similar to those obtained in untreated WT mice.

Conclusion: Our findings pointed out the rectal mucosa as an additional target tissue to study in vivo ion transport abnormalities. The RPD test discriminates between CF and non-CF and can be used to investigate the efficacy of therapeutic strategies to rescue CFTR function. As for the airways, vardenafil restores chloride secretion across the GI epithelium. Immunolocalization of CFTR protein in colon tissue preparations are under investigation in KO, CF and WT mice treated or not-treated with vardenafil.

Pathological role of the calpain/calpastatin system in cystic fibrosis

M. Averna, L. Minucci, S. Palena, F. Cresta, S. Pontremoli, E. Melloni. 1University of Genoa, DIMES, Genoa, Italy; 2University of Genoa, CF Center Pediatric Department Institute G. Gaslini, Genoa, Italy

Objectives: To establish the involvement of the calpain/calpastatin system, the components of the calcium-dependent proteolysis, in cystic fibrosis (CF), we have analyzed peripheral blood mononuclear cells (PBMC) of 12 CF patients (F508del-CFTR homozigotes) in which the alteration in levels and localization of F508del-CFTR is identical to that reported in airway epithelial cells models.

Methods: The role of calpain in CF has been studied by immunoblot analysis, immunoprecipitation, confocal microscopy, and assay of intracellular protease activity.

Results: Whereas in PBMC from controls, the basal calpain activity is almost undetectable, in CF-PBMC the protease activity is significantly measurable, due to an increase in [Ca2+], and a decrease in the level of both calpastatin protein and inhibitory efficiency. The imbalance in regulation of the calpain/calpastatin system favors the activation of the protease, explaining the presence of the digested CFTR form. As a result of such protease activation, NHERF-1, a partner of CFTR in its functional complexes, is also present in PBMC of CF-patients in a digested form, showing a mass of 20 kD. This covalently modified NHERF-1 is completely absent in controls as well as in heterozygous CF parents. Ezrin, another component of the CFTR functional clusters, is also degraded in CF PBMC.

Conclusion: Our observations are indicating that calpain digests different components of the CFTR-generated protein complexes, promoting their removal from plasma membranes and accumulation into cytoplasm. Restoration of the intracellular calpain regulation with new synthetic inhibitors could provide a new therapeutic approach to CF.