"Topical eye treatment with p-blocker abolishes sweat secretion triggered by intradermal isoprenaline plus aminophylline: a clinical observation"

Leal, Teresinha ; Noël, Sabrina ; Bergamini, Gabriella ; Calcaterra, Elisa ; Sorio, Claudio ; Nguyen-Khoa, Thao ; Melotti, Paola

ABSTRACT

Objectives: Two new versions of the sweat test have recently been developed with claimed advantages of being sensitive enough to quantify residual CFTR function and to measure efficacy of basic therapies.

Methods: Rates of water evaporation (kg water/hr/m2) and of volume of sweat per gland (nL/min) were measured in a healthy female, 61 yrs, following the reported test protocols. Results: Cholinergic stimulation gave normal results (maximal evaporimetric response 5 min after intradermal injection of carbachol: 95.1 kg water/hr/m2 and gland secretion rate obtained 10 min after methacholine injection: 1.8 nL/min). However, the β-adrenergic (isoprenaline plus aminophylline) stimulation, induced in the presence of atropine to block cholinergic stimulation, was abolished (β-adrenergic/cholinergic ratio: 0 and 5.8% of the normal values for evaporimetry and bubble test, respectively), eliciting a diagnosis of CF. The results were confirmed by repeating each test at least twice. Sweat chlo...

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Nasal potential difference in young children is feasible

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Objectives: The diagnosis of cystic fibrosis is based on characteristic clinical symptoms and either a positive sweat test or identification of related mutations on exons of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene alleles. A significant number of patients have no definite diagnosis, having possibly related symptoms, borderline sweat test and no identifiable known mutation on the CFTR gene. Nasal Potential Difference (NPD) is an established method of diagnosis in these patients. Until now standard values for very young patients have not been developed and validated. The aim of this study was to evaluate the feasibility of NPD in young children.

Methods: The standard protocol for NPD was adapted for young children. In the standard protocol infusions of amiloride, chloride-free and isoproterenol are for 3 minutes. In the current protocol this was reduced to 2 minutes so the entire test is completed in 10 minutes.

Results: 35 children aged 2 months to 3 years of age were enrolled in the study. Diagnoses included Failure to thrive (15), chronic diarrhea (6), respiratory problems (10), meconium plug (2). Mean sweat test was 53mmol/L. The NPD was abnormal ($\Delta$amil/A$\Delta$Am < 0.7 was defined as an abnormal NPD) in 24% of the children and no identifiable known mutation on the CFTR gene. Nasal Potential Difference (NPD) is an established method of diagnosis in these patients. The procedure was tolerated in all children.

Conclusion: NPD is feasible in young children and may be useful in the diagnosis of CF in questionable cases.

Topical eye treatment with $\beta$-blocker abolishes sweat secretion triggered by intradiscal isoprenaline plus aminophylline: a clinical observation

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Objectives: Two new versions of the sweat test have recently been developed with claimed advantages of being sensitive enough to quantify residual CFTR function and to measure efficacy of basic therapies.

Methods: Rates of water evaporation (kg water/hr/m2) and of volume of sweat per gland (nL/min) were measured in a healthy female, 61 yrs, following the reported test protocols.

Results: Cholinergic stimulation gave normal results (maximal evaporimetric response 5 min after intradermal injection of carbachol: 95.1 kg water/hr/m2 and gland secretion rate obtained 10 min after methacholine injection: 18 nL/min). However, the $\beta$-adrenergic (isoprenaline plus aminophylline) stimulation, induced in the presence of atropine to block cholinergic stimulation, was abolished ($\beta$-adrenergic/cholinergic ratio: 0 and 5.8% of the normal values for evaporimetry and bubble test, respectively), eliciting a diagnosis of CF. The results were confirmed by repeating each test at least twice. Sweat chloride after pilocarpine iontophoresis using a coulometric method was 24 mmol/L. PCR-based screening showed no CFTR mutation. History of current medications possibly interfering with the pharmacological agents used during the tests revealed long-term (>12 yrs) treatment of a well-controlled primary angle closure glaucoma consisting on a daily topical use of $\beta$-blocker cartol 2% (1 drop/left eye). No apparent symptom of $\beta$-blocker intoxication is present (heart rate: 66 beats/min, regular sinus rhythm, no symptom of asthma).

Conclusion: Much attention should be paid to the use of medications that may interfere with the pharmacological steps of the $\beta$-secretion tests. The clinical observation confirms the proof-of-concept that the second phase of both tests directly evaluates $\beta$-adrenergic sweat production that is very sensitive to $\beta$-blocker treatment, even at topical eye use, leading to false positive test results.

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Cell Biology/Physiology/New therapies

Successful gene editing of human embryonic stem cells to generate a novel CF airway epithelial model

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Introduction: Primary human airway epithelial cultures have enabled huge advances in CF therapeutics, such as Kalydeco and Orkambi. However, further cystic fibrosis (CF) drug discovery is still impeded by the limited availability and high background variability of these cells between CF donors.

Objectives: To develop a novel CF epithelial cellular model for CF drug discovery.

Methods: We have used TALENs to genetically modify human embryonic stem cells (hESCs) and a single-stranded oligodeoxynucleotide (ssODN) to introduce the F508del mutation.

Results: We have generated a TALEN targeting the CFTR-gene (CFTR- TALEN), which introduces a double strand break (DSB). Correct assembly was confirmed by restriction digestion and sequencing. To promote successful genome editing, hESCs were adapted to grow as single-cells under feeder-free conditions. To improve the DNA plasmid delivery into hESCs, lipid-based transfection reagents, electroporation and nucleofection were tested using eGFP. Transfection efficiency ranged from 9% to 90% as assessed by flow cytometry, with nucleofection being the most efficient. Transfection of the CFTR- TALEN was confirmed by immunofluorescence and 15% CFTR- TALEN activity was detected by the T7 endonuclease I assay, in hESCs. In order to repair the DSB and introduce the desired mutation, a F508del-ssODN was designed and transfected with the CFTR- TALEN into hESCs. PCR analysis and sequencing showed integration of the F508del mutation at the correct site within the pool of transfected hESCs.

Conclusion: hESCs have been successfully modified using CFTR- TALEN and ssODN. The modified and isogenic control hESCs will be selected, expanded and differentiated into fully polarised airway epithelial cell cultures using a recently described approach (Wong et al, 2015), and fully characterized biochemically and functionally. The gene edited hESCs may also be used to model other CF affected tissues.

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Validating the organoid model across European laboratories

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Objectives: The intestinal organoid model can be used to test patient-specific residual CFTR function and predict patient-specific drug response.