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Glutathione S-transferases related to *P. aeruginosa* lung infection in cystic fibrosis children: Preliminary study

Marie-Noëlle Feuillet-Fieux [a,*], Thao Nguyen-Khoa [a], Marie-Anne Loriot [b,c], Mairead Kelly [a], Philippe de Villartay [d], Isabelle Sermet [d], Patricia Verrier [d], Jean-Paul Bonnefont [e], Philippe Beaune [b,c], Gérard Lenoir [d], Bernard Lacour [a]

[a] *Assistance Publique-Hôpitaux de Paris, Necker-Enfants Malades Hospital, Laboratory of Biochemistry A, 149 rue de Sèvres, 75015 Paris, France*
[b] *European Georges Pompidou Hospital, Laboratory of Biochemistry, 75015 Paris, France*
[c] *INSERM U-775, René Descartes University, 45 rue des Saints-Pères, 75006 Paris, France*
[d] *Department of Pediatrics, Necker-Enfants Malades Hospital, 149 rue de Sèvres, 75015 Paris, France*
[e] *Department of Genetics, Necker-Enfants Malades Hospital, 149 rue de Sèvres, 75015 Paris, France*

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Abstract

Objectives: In cystic fibrosis (CF) children, we investigated the predictive impact of glutathione S-transferases (GST) activity and genotypes P1, M1 and T1, and antioxidant levels on stage-severity of *Pseudomonas aeruginosa* lung infection.

Methods: GST activity was determined in whole blood by spectrophotometry, and GST genotypes by multiplex PCR RFLP for 36 CF and 9 control children. Levels of glutathione in erythrocyte and vitamins A, E and C in plasma were measured by HPLC.

Results: No difference in GST activity and no relationship between GST activity and antioxidant levels were observed in CF children as compared to controls. However, GST activity was lower in CF children with severe clinical status and infection, and the frequency of GSTP1 wild type genotype AA, prevalent in uninfected CF children (75%), decreased in infected ones (33%).

Conclusion: GST activity and genotype could play an important role in modulating *P. aeruginosa* lung infection in CF patients.

Keywords: Cystic fibrosis; Glutathione S-transferase; Oxidative stress; Lung infection

Introduction

Cystic Fibrosis (CF) is the most common inherited autosomal recessive disorder among Caucasians caused by mutations in the gene encoding the CF Transmembrane Conductance Regulator (CFTR). The first *Pseudomonas aeruginosa* (*P. aeruginosa*) infection is the worsening CF clinical event, leading to oxidative tissue damage and lung dysfunction.

Glutathione S-transferases (GST) are ubiquitous multifunctional enzymes which play a key role in cellular detoxification. They protect cells against oxidative stress by catalysing reduced glutathione (GSH) conjugation with toxic electrophilic xenobiotics and carcinogens. GST activity depends on its genotype and the presence of GST polymorphisms can lead to decreased activity [1], thus generating an oxidative stress at the origin of the severity of pulmonary infection.

It is known that GSH is transported through the CFTR of epithelial cells, particularly in the lung airway lining fluid, in which its concentration was found 50 times higher than in plasma [2,3]. CFTR mutations, causing a canal dysfunction, lead to a deficiency of cellular GSH efflux, thus decreasing GSH levels in bronchial surfactant affecting its antioxidant capacity and its antibacterial properties [4].

The aim of this study was to evaluate levels of antioxidant systems as predictive factors of the severity of *P. aeruginosa* lung infection. Our objectives were to assess the predictive interest of GST activity and/or genotype in the earlier risk of

* Corresponding author. Laboratory of Biochemistry A, Necker-Enfants Malades Hospital, AP-HP, 149 rue de Sèvres, 75743 PARIS Cedex 15, France. Fax: +33 1 4449 5120.
E-mail address: marie-noelle.feuillet@nck.aphp.fr (M-N Feuillet-Fieux).

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P. aeruginosa infection. We investigated the relationship between GST activity and antioxidant status, evaluated by erythrocyte GSH and plasma vitamin A, E and C, and the clinical and lung infection status of the children at blood collection.

Patients and methods

Patients

The study group consisted of 45 Caucasian children (21 girls and 24 boys) whose parents gave written informed consent. Thirty six CF children and 9 healthy controls were included in the study and are described in Table 1. The protocol was approved by the Necker-Enfants Malades ethical committee. Blood samples were collected using Vacutainer® tubes (Becton Dickinson), one of 2 mL containing EDTA and one of 3 mL containing heparinate as anticoagulant.

Clinical data including spirometric parameters (volume expiratory maximal in 1 s (VEMS), force expiratory volume in 1 s (FEV1), force vital capacity (FVC)), nutritional status (height and weight, body mass index), Shwachmann score and sputum *P. aeruginosa* testing, were gathered to evaluate the general clinical status at blood collection. Thirty two (89%) presented pancreatic exocrine insufficiency. Among these 36 CF children, 29 (80%) carried the most common CFTR mutation F508del (18 (50%) homozygous and 11 (31%) heterozygous) and 7 (19%) carried other mutations. Twenty one presented one or two other typical features: 4 had meconial ileus (MI, 11%) one of which also carried digital hippocratism (DH); 8 had nasal polyposis (NP, 22%) of which 3 had also DH; and 13 DH (36%) of which one had also MI and 3 had NP (Fig. 1). The repartition of these feature associations in our CF children correspond to the well known distribution [5–8]. Twelve children were chronically infected with *P. aeruginosa* ("present infected"), twelve had presented precocious *P. aeruginosa* infection, well eradicated at blood collection ("past infected") and the other twelve were free of this germ ("never infected").

Antioxidant parameters

Erythrocyte glutathione levels were determined as previously described [9]. Briefly, after deproteinization by 5% metaphosphoric acid, separation was performed by High Performance Liquid Chromatography (HPLC) with coulometric detection. Results are expressed as GSH levels and as GSSG/GSH ratio, GSSG being expressed as GSH equivalent (1 mole of GSSG corresponding to 2 equivalents of GSH).

Table 1
Characteristics of the 45 Caucasian children included in the study

		All	Girls	Boys
CF patients	Number	36	18	18
	Mean age (years)	10.2±5.1	10.3±4.5	10.1±5.7
	Range (years)	2.5–21	4–16	2.5–21
Controls	Number	9	3	6
	Mean age (years)	8.3±2.2	7.1±3.9	9.3±1.0
	Range (years)	3–11	3–10.5	8–11

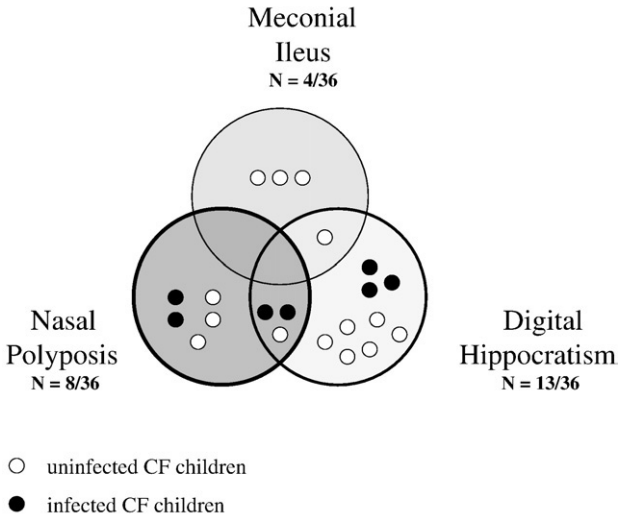

Fig. 1. Clinical sign association in 21 out of 36 CF children related with *Pseudomonas aeruginosa* infection.

Plasma levels of vitamin C, as its reduced form ascorbate, were determined by HPLC associated with coulometric detection as described previously [10]. Plasma levels of vitamin A and E were measured by HPLC using UV detection, at 325 nm for vitamin A and 292 nm for vitamin E as previously described [9].

GST activity

Preparation of reagents

Assay buffer, a 100 mM potassium phosphate pH 5.5, containing 0.1% Triton X-100 was used to prepare sample buffer with addition of 1 mM GSH (Sigma-Aldrich, St. Louis, MO, USA) and BSA 1 mg/mL (Sigma). The GST control, a solution of rat liver GST (Cayman Chemical Company, An Arbor, MI, USA), and the GST samples were diluted in sample buffer prior to assaying. GSH 10 mM in HPLC-grade water and GST control solutions were stored at −80 °C until the assay. 1-chloro-2,4-dinitrobenzene (30 mM CDNB, Sigma) ethanolic solution was stored at −20 °C.

Preparation of hemolysate

One milliliter of whole blood samples was lysed with one volume of ice-cold HPLC-grade water. Then, it was diluted to yield a 1/20 hemolysate in sample buffer. This operation was carried out at 4 °C.

Whole blood enzyme assay

Total GST activity was evaluated with a sensitive and reproducible procedure without purification steps. The GST assay measured total GST activity by conjugation of CDNB with GSH according to a modified version of that described in cell homogenates by Habig et al. [11]. Total GST activity was measured in whole blood samples similarly to a previous serum protocol [12]. Briefly, 50 µL of CDNB (1.5 mM), 100 µL of