Photoacoustic analysis of the solubilization kinetics of pulmonary secretions from cystic fibrosis patients – secretor and non-secretor phenotypes

P.R.Barja, C.C.Coelho, R.F.Paiva, M.A.Barboza, L.C.Matos, C.C.B.Matos, and L.V.F.Oliveira

Research and Development Institute, UNIVAP, Av. Shishima Hifumi 2911, São José dos Campos, SP, Brasil
Faculty of Medicine of São José do Rio Preto (FAMERP), São José do Rio Preto, SP, Brasil
Rehabilitation Sciences Master's Program, Nove de Julho University (UNINOVE), São Paulo, SP, Brasil
barja@univap.br

Abstract. Cystic fibrosis (CF) is an autosomal recessive inherited disease that increases viscoelasticity of pulmonary secretions. Affected patients are required to use therapeutic aerosols continuously. The expression of ABH glycoconjugates in exocrine secretions determines the nature of part of the carbohydrates present in these secretions, allowing the classification of individuals into the so-called “secretor” and “non secretor” phenotypes. The aim of this work was to employ photoacoustic (PA) measurements to monitor the solubilization kinetics of pulmonary secretions from CF patients, analyzing the influence of the secretor status in the solubilization kinetics of samples nebulized with different therapeutic aerosols. Sputum samples were obtained by spontaneous expectoration from positive and negative secretor CF patients. Each sample was nebulized with i) tobramycin, ii) alpha dornase, and iii) N-acetylcysteine in a PA cell; fitting of the data with the Boltzmann equation led to the determination of $t_0$ (typical interaction time) and $\Delta t$ (solubilization interval) for each curve. Differences between the secretor and non-secretor phenotypes were statistically significant in the groups for tobramycin and alpha dornase, but not for N-acetylcysteine. Results show that the secretor status influences the solubilization of pulmonary mucus of CF patients nebulized with tobramycin and alpha dornase.

1. Introduction
Cystic fibrosis (CF) is a monogenic, autosomal recessive inherited disease resulting from alterations of the cystic fibrosis transmembrane conductance regulator gene (CFTR), leading to a considerable increase in the viscoelasticity of pulmonary secretions. This leads to obstruction of the airways by mucus [1-3]; CF patients are thus required to use therapeutic aerosols to solubilize and facilitate expectoration [4].

ABH glycoconjugates are structurally related to antigens which define ABO blood groups. The expression of ABH glycoconjugates in exocrine secretions (controlled by the enzyme $\alpha$-2-L-
fucosiltransferase (FUTII) encoded by FUT2 gene) determines the nature of part of the carbohydrates present in these secretions, thus altering their biochemical properties and allowing the classification of individuals into the so-called “secretor” and “non secretor” phenotypes [5, 6]. The influence of the secretor and non secretor phenotypes on the solubilization of the pulmonary mucus treated with therapeutic aerosols in order to reduce the viscoelasticity is still unknown.

In photoacoustic (PA) measurements, the signal depends on the optical and thermal properties of the sample. When the sample undergoes changes in its composition or structure, the production and propagation of heat upon radiation is modified, thereby altering the PA signal. The photoacoustic (PA) technique has been employed to study the interaction between therapeutic aerosols and secretions, allowing the determination of the typical time between the aerosol and the secretion ($t_0$) and the solubilization period ($\Delta t$). Differences in the absorption times of isotonic saline solution were observed in the sputum of CF patients with and without pneumonia using the PA technique, also used to monitor the interaction time of sputum of CF patients using hypertonic saline solutions and N-acetylcysteine [7].

The aim of the present study was to employ PA measurements as a function of time to characterize the solubilization kinetics of pulmonary secretions from CF patients, analyzing the influence of the secretor status in the solubilization kinetics of samples nebulized with different therapeutic aerosols.

2. Materials and Methods

Of the ten CF patients (between 10 and 29 years-old) selected, 6 are regularly treated in the CF Reference Center of the Regional Foundation of Medicine School in São José do Rio Preto (FUNFARME) and 4 in the CF Reference Center of the State University in Campinas (UNICAMP). Definition of the secretor and non secretor phenotypes was achieved by genotyping the FUT2 (G428A) gene using PCR-RFLP according to the protocol of Svensson and colleagues [8]. CF was confirmed for all the 6 positive and 4 negative secretor patients by positive sweat test (considered the gold standard). Sputum samples were obtained by spontaneous expectoration, following the protocol of Bossi [9]; each sample was placed in a polystyrene tube lubricated with liquid Vaseline to avoid dehydration and stored at -20ºC until PA analysis.

PA measurements employed a tungsten lamp (24V, 250W) as light source, modulated at 17Hz by a mechanical chopper (Stanford Research Systems, mod.SR540). The chopper and the electret microphone of the cell were connected to a lock-in amplifier (Stanford Research Systems, mod.SR530) used to capture the PA signal, sent to a microcomputer that controlled the data acquisition process.

Before PA measurements, each sample was naturally thawed at room temperature and subsequently submersed in xylol for five seconds to remove the Vaseline; each sample was then divided in three portions with volumes of 0.1mL. Initially, each sample was evaluated for 5 minutes (before applying aerosol) to measure the baseline PA signal. Subsequently, each sample portion was nebulized with one of the aerosols: i) tobramycin, ii) alpha dornase, and iii) N-acetylcysteine (utilized at doses as recommended by the fabricants for clinical use). The solubilization process was evaluated through PA measurements as a function of time. Each PA data curve was adjusted by a sigmoidal curve, Boltzmann type (Origin 7.0®):

$$PA(t) = \frac{A_1}{1 + e^{\frac{t-t_0}{\Delta t}}} + A_2$$

(eq.1)

where PA(t) is the PA amplitude at time t, $A_1$ and $A_2$ are the baseline and final amplitudes of the PA signal, $t_0$ is the time to reach the maximum rate of change in the process and $\Delta t$ the effective time interval corresponding to the solubilization process. Data was stored in the computer; adjustment curves were produced by the Microcal Origin 7.0® software and statistical analysis employed the GraphPad Instat 3.0® software.
3. Results and discussion

Table 1 shows the values obtained for $t_0$ and $\Delta t$, for each aerosol and secretor state.

| Aerosol          | Secretors | Non-secretors | p-value |
|------------------|-----------|---------------|---------|
| Tobramycin       | $t_0$     | 8.8 ± 5.2     | 13.7 ± 1.4 | 0.03   |
|                  | $\Delta t$| 4.8 ± 2.4     | 4.9 ± 1.2 | 0.88   |
| Alpha dornase    | $t_0$     | 10.6 ± 5.2    | 8.1 ± 2.6 | 0.35   |
|                  | $\Delta t$| 2.7 ± 1.6     | 4.8 ± 1.5 | 0.04   |
| N-acetylcysteine | $t_0$     | 8.4 ± 4.3     | 12.4 ± 6.6 | 0.35   |
|                  | $\Delta t$| 2.4 ± 2.0     | 2.4 ± 2.1 | 0.99   |

Nebulization with tobramycin produced $\Delta t$ similar for both secretor and non secretor phenotypes, but the mean value of $t_0$ was significantly higher for non secretor phenotype ($p=0.03$). Nebulization using alpha dornase also presented statistically significant differences between secretor and non secretor phenotypes, but in $\Delta t$ (also higher for non secretor phenotype, with $p=0.04$). Both for tobramycin and alpha dornase, non secretors also presented greater homogeneity in the solubilization process (lower standard deviations observed for $t_0$ and $\Delta t$). The N-acetylcysteine aerosol produced solubilization equivalent for both phenotypes.

3.1. Tobramycin

The results of this study suggest that tobramycin is more effective (lower $t_0$) in the solubilization of sputum in secretors. Tobramycin is an aminoglycoside antibiotic commonly utilized in CF patients for the treatment of infections by *Pseudomonas aeruginosa*; its pharmacodynamic effect also contributes to solubilization of the pulmonary mucus [10-12]. One study that analyzed the binding of antibiotics to the sputum of CF patients reported that the degree of binding of tobramycin is dependent on the concentration of macromolecules in the secretion [12]. More recently, it was observed that the pattern of glycosylation of MUC5B is dependent on the ABO blood group and secretor phenotype [13]. It is possible that ABH oligosaccharides of secretors contribute to the interaction of tobramycin with sputum. The results indicate a reduction in the typical interaction time of solubilization for carriers of secretor phenotype.

3.2. Alpha dornase

Alpha dornase is a human recombinant deoxyribonuclease that reduces the viscoelasticity of the sputum by means of fragmentation of high molecular weight DNA molecules released by infiltrating neutrophils. This inhalation drug, used daily by CF patients to facilitate mucus clearance, has hydrosoluble properties [14]. PA measurements indicated that the solubilization interval of the sputum was influenced by the secretor status, suggesting that this drug remain more time acting on the sputum from non secretor phenotypes. The ABH glycoconjugates present in the sputum of secretor phenotype carriers are derived from the type 1 precursor oligosaccharides which are fucosylated by FUTII enzyme [15]. Carriers of non secretor phenotypes do not express the same diversity of ABH glycoconjugates, as they do not have the functional FUTII enzyme, due to the mutations that occur in exon 2 of the *FUT2* gene, which inactivate its active site [8]. Thus, the Type 1 precursor oligosaccharidic chains of these individuals tend to present longer and non-diversified structures compared to those that occur in secretors [16]. The structural differences in the ABH glycoconjugates resulting from the joining action of *FUT2* and *A* and *B* genes may cause variations in the polarity and hydrosolubility of the sputum from secretors and non secretors, influencing the solubilization interval with alpha dornase. Actually, analysis of ABH glycolipids extracted from small intestine secretions revealed the presence of different fractions of these glycoconjugates resulting from the diversity of
oligosaccharidic chain lengths [17]. Therefore, it is possible that the structural and chemical variability of the oligosaccharidic chains of non secretor patients contributes to the greater interaction of alpha dornase with the sputum due to the hydro soluble properties of this drug.

3.3. N-acetylcysteine

PA analysis of the sputum of CF patients indicated that the secretor status does not influence the solubilization kinetics for nebulization with N-acetylcysteine. N-acetylcysteine is derived from the cysteine amino acid; it has a tiol reducing property which favors cleavage of the polypeptidic chains that constitute the mucins of sputum [18]. This process does not seem to be influenced by the presence of oligosaccharidic portions of the ABH glycoconjugates in the mucins as a result of glycosylation controlled by the FUT2 and ABO genes. The observation that the solubilization kinetics for nebulization with N-acetylcysteine does not depend on the secretor status could be expected, because the proteic portions of the mucins do not seem to differ between positive and negative secretors.

4. Conclusion and perspectives

As assessed by PA measurements, solubilization curves for pulmonary secretions of CF patients can be well described through a Boltzmann curve. Measurements indicate that the solubilization process requires about 15 minutes to be effective. Results show that the secretor status influences the solubilization process of pulmonary mucus of CF patients nebulized with tobramycin and alpha dornase, but not with N-acetylcysteine. These results are expected to contribute to the improvement of the design and prescription of therapeutic aerosols to CF patients according to their secretor/non secretor phenotypes.

References

[1] M.J.Welsh, L.C.Tsui, T.F.Boat, A.L.Beaudet. Cystic fibrosis. In: Scriver CR, Sly WS, editors. The metabolic and molecular bases of inherited disease. 7th ed. New York: McGraw-Hill, p.3799-878 (1995).
[2] F.J.C.Reis, N.Damasceno. J.Pediatr.(Rio J.) 74 (Supl.1), S76-S94 (1998).
[3] S.Raskin, J.A.Phillips, M.R.Krishnamani et al. Am.J.Med.Genet. 46(6), 665-9 (1993).
[4] R.Tarran. Proc.Am.Thorac.Soc. 1(1):42-6 (2004).
[5] M.A.I.Barboza, C.C.B.Mattos, P.R.Barja, L.V.F.Oliveira, L.C.Mattos. Arch.Med.Science 4, 218-223 (2008).
[6] S.M.Henry. Transfus.Clin.Biol. 8(3), 226-30 (2001).
[7] F.L.Dumas, F.R.Marciano, L.V.Oliveira et al. Med.Eng.Phys. 29(9), 980-3 (2007).
[8] L.Svensson, A.Petersson, S.M.Henry. Transfusion 40(7), 856-60 (2000).
[9] R.Bossi. Methods for collecting and measuring mucus in humans. In: Braga PC, Allegra L, editors. Methods in bronchial mucology. New York: Raven Press, p.13-20 (1988).
[10] W.H.Beggs, F.A.Andrews. J.Infect.Dis. 134(5), 500-4 (1976).
[11] C.R.Bodem, L.M.Lampton, D.P.Miller, E.F.Tarka, E.D.Everett. Am.Rev.Respir.Dis 127(1), 39-41 (1983).
[12] R.Ramphal, M.Lhermitte, M.Filliat, P.Roussel. J.Antimicrob.Chemother. 22(4), 483–90 (1988).
[13] K.A.Thomsson, B.L.Schulz, N.H.Packer, N.G.Karlsson. Glycobiology 15(8), 791-804 (2005).
[14] S.Shak, D.J.Capon, R.Hellmiss, S.A.Marsters, C.L.Baker. Proc Natl Acad Sci USA 87(23), 9188-92 (1990).
[15] R.Oriol. ABO Hh, Lewis and Secretion: serology, genetics and tissue distribution. In: Cartron JP, Rouger P. Blood cell biochemistry: molecular basis of human blood group antigens. N.York: Plenum, p.37-73 (1995).
[16] J.Angström, T.Larsson, G.C.Hansson et al. Glycobiology 14(1), 1-12 (2004).
[17] S.Henry, P.A.Jovall, S.Ghardashkhani et al. Glycoconj.J. 14(2), 209-23 (1997).
[18] G.P.Ventresca, V.C.Ferrari. Acetylcysteine. In: Braga PC, Allegra L, editors. Drugs in bronchial mucology. 2nd ed. New York: Raven Press, p. 77-102 (1989).