

Thiocyanate concentration in saliva of cystic fibrosis patients

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Abstract: Thiocyanates (SCN⁻) are ubiquitous in nature. There are indispensable part of host defense system that act as a substrate for lactoperoxidase (LPO). In our study we present initial data on SCN⁻ concentration in saliva of CF patients in comparison to healthy non-smokers and healthy smokers. 5 ml of saliva was collected from each subject to a sterile tube and thiocyanate concentration was measured in each sample. The results of the measurements are presented on Fig. 1. Mean concentration of SCN⁻ in saliva of CF patients was 0.031 ± 0.0052 g/l, in healthy non-smokers 0.039 ± 0.0048 g/l and in healthy smokers 0.048 ± 0.0161 g/l. The differences between each group were statistically significant. Studies on larger group of patients and probably on different material (BALF or induced sputum) should present interesting data complementing the in vitro studies.

Key words: Cystic fibrosis - Thiocyanate - Saliva

Thiocyanates (SCN⁻) are ubiquitous in nature. There are indispensable part of host defense system that act as a substrate for lactoperoxidase (LPO). LPO oxidize airway surface liquid SCN⁻ thereby generating antimicrobial agent hypothiocyanite (OSCN⁻) [1-3]. The OSCN⁻ formation is believed to be necessary for elimination of bacteria from airway mucosal surfaces [4]. Recently publications report on impairment of this novel host defense system in cystic fibrosis patients. In a study on human and rat airway epithelia on cow tracheal explants Moskwa *et al.* [4] showed that CF epithelia failed to excrete SCN⁻ and thus this system is

In our study we present initial data on SCN⁻ concentration in saliva of CF patients in comparison to healthy non-smokers and healthy smokers. We decided to include the healthy smokers as an additional control group as it was previously shown that in this group SCN⁻ levels in saliva are elevated [5].

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Materials and methods

Patients. The study was conducted in three groups of patients CF patients, healthy non-smokers and healthy smokers. The groups were sex and age matched. In CF patients delF508 was present in 13 (65%) alleles. Other mutations were G542X - 2 alleles (10%), R553X - 2 alleles (10%), 2134delT - 1 allele (5%), 3849+10kb C-T - 1 allele (5%), one mutation was undefined.

Saliva samples. 5 ml of saliva was collected from each subject to a sterile tube and the SCN⁻ concentration was measured within 2 h after collection.

Thiocyanate designation. To 1 ml of whole saliva 0.03 ml of 2 M HCl and 0.03 ml of 5% FeCl₃ was added. Each sample was thoroughly mixed and estimated towards to control samples (1 ml H₂O, 0.03 ml of 2 M HCl and 0.03 ml 5% FeCl₃) at spectrophotometer at wave length of $\lambda=570$ nm.

Thiocyanate concentration was estimated according to calibration plot prepared using standard solutions of NH₄SCN. The data from obtained from the calibration plot were calculated according to the equation:

$$\text{SCN}^-[g/l] = \frac{[\text{concentration from the plot}] \times [\text{SCN}^- \text{ molar mass} = 58 \text{ g/mol}]}{[\text{NH}_4\text{SCN molar mass} = 76 \text{ g/mol}]}$$

Statistical analysis. The differences between mean were calculated using student t-test.

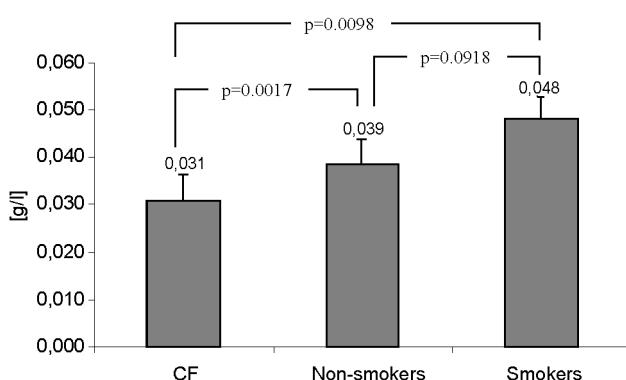


Fig. 1. The concentration of SCN⁻ in saliva of CF patients, healthy non-smokers and healthy smokers.

Results and discussion

The results of the measurements are presented on Fig. 1. Mean concentration of SCN⁻ in saliva of CF patients was 0.031 ± 0.0052 g/l, in healthy non-smokers 0.039 ± 0.0048 g/l and in healthy smokers 0.048 ± 0.0161 g/l. The differences between groups were statistically significant (Fig. 1) although the studied groups may be considered to small. The saliva was chosen as study material as it is one of the major sites of active LPO and is fairly easy to obtain [6].

The impaired LPO function and differed thiocyanate concentration was described by Azen [7] and this finding were confirmed in several recent studies [8]. Childers *et al.* reviewed a problem of impaired glutathione transport and its thiocyanate conjugates in cystic fibrosis patients [9]. SCN⁻ ions were used as probe of Cl⁻ channel pores since 1960s. It was shown that CFTR channel is permeable to thiocyanates [9,10]. *In vivo* studies are necessary to estimate exact lack of thiocyanates in cystic fibrosis patients in sites where LPO is active. Here we present preliminary results of the study on SCN⁻ concentration in a small group of cystic fibrosis patients. Studies on larger group of patients and probably on different material

(BALF or induced sputum) should present interesting data complementing the *in vitro* studies.

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References

- [1] Reiter N, Perraudeau JP. Lactoperoxidase: Biological function. In: *Peroxidases in Chemistry and Biology*, vol I, eds. Everse J, Everse KE, Grisham MB. CRC Press. Boca Raton. 1991; pp. 143.
- [2] Conner GE, Salathe M, Forteza R. Lactoperoxidase and hydrogen peroxidase metabolism in the airway. *Am J Respir Crit Care Med.* 2002;166:S57-S61.
- [3] Weuffen W, Bergman H, Blohm H, Bohland H, Hiepe Th, Schonfeld P. Thiocyanate - a biological active ion of veterinary and medical relevance. *Berl Munch Tierarztl Wschr* 2003;116:144-156.
- [4] Moskwa P, Lorentzen D, Excoffon KJDA, Zabner J, MacCray Jr PB, Nauseef WM, Dupuy C, Banfi B. A novel host defense system of airways is defective in cystic fibrosis. *Am J Respir Crit Care Med.* 2007;175:174-183.
- [5] Morabia A, Bernstein MS, Curtin F, Berode M. Validation of self-reported smoking status by simultaneous measurement of carbon monoxide and salivary thiocyanate. *Prev Med.* 2001; 32(1):82-8.
- [6] Fragozo MA, Fernandez V, Forteza R, Randell SH, Salathe M, Conner GE. Transcellular thiocyanate transport by human airway epithelia. *J Physiol.* 2004;561:183-194.
- [7] Azen EA. Salivary peroxidase activity and thiocyanate concentration in human subjects with genetic variants of salivary peroxidase. *Arch Oral Biol.* 1978;23:801-805.
- [8] Gerson C, Sabater J, Scuri M, Torbat A, Coffey R, Abraham JW, Lauredo I, Forteza R, Wanner A, Salathe M, Abraham WM, Conner GE. The lactoperoxidase system functions in bacterial airway clearance. *Am J Respir Cell Mol Biol.* 2000; 22:665-671.
- [9] Childers M, Eckel G, Himmel A, Caldwell J. A new model of cystic fibrosis pathology: lack of transport of glutathione and its thiocyanate conjugates. *Med Hypoth.* 2007;68:101-112.
- [10] Linsdell P. Thiocyanate as a probe of the cystic fibrosis transmembrane conductance regulator chloride channel pore. *Can J Physiol Pharmacol.* 2001;79:573-579.

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