EDITORIAL

Pseudomonas aeruginosa and cyanide production

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Infection and subsequent chronic infection of the cystic fibrosis (CF) lung with organisms such as *Pseudomonas aeruginosa* probably occurs at an early stage in the life of a child suffering from CF. Clinicians worldwide are eager to isolate the organism as early as possible in an attempt to treat the patient before any permanent damage occurs and hopefully to eliminate the organism completely from the respiratory tract for many years. Young children are usually unable to expectorate sputum for microbiological analysis and cough swabs are relatively insensitive. Induced sputum techniques are generally not well tolerated in children and bronchoscopic manoeuvres are very invasive, requiring a general anaesthetic. There has, therefore, been interest in discovering whether there are any surrogate markers which could be helpful as a specific indication of the presence of *P. aeruginosa*. It has been postulated that cyanide could be one such compound.

P. aeruginosa is one of a limited number of organisms which can synthesise cyanide [1]. Synthesis occurs by the oxidative decarboxylation of glycine by a hydrogen cyanide synthase enzyme. This process also produces four electrons and four hydrogen ions per glycine molecule. Cyanide synthesis by *P. aeruginosa* occurs under microaerobic ($O_2 < 5\%$) conditions but is inactivated under both atmospheric oxygen and strictly anaerobic conditions [1, 2]. Cyanide production by *P. aeruginosa* is maximised at temperatures between 34°C and 37°C [3]. Studies which have investigated cyanogenesis in relation to *P. aeruginosa* using liquid or solid samples have assayed cyanide concentrations using a cyanide electrode. Studies analysing gas samples have measured the volatile compound hydrogen cyanide (HCN) using mass spectrometry techniques.

In *P. aeruginosa* cultures the cyanide level has been shown to reach 300–500 μM [1–3] and in the headspace over the cultures the HCN concentration has reached 17 ppm [4]. Cyanide has also been identified in the sputum [5, 6] and HCN in the breath of CF and non-CF bronchiectasis patients infected with *P. aeruginosa* [5, 7]. Conversely, cyanide is absent from the sputum of bronchiectasis patients without *P. aeruginosa* infection [5, 6] and HCN is very low or absent from the breath of healthy children [8]. Studies in healthy adults have shown that much of the HCN is generated in the mouth and this could have importance when measuring HCN in exhaled breath in relation to infection with *P. aeruginosa* [9].

The biological reason for the presence of cyanide in association with *P. aeruginosa* is unclear but it is likely that it provides *P. aeruginosa* with an advantage in the range of ecological niches it inhabits and may, therefore, have a role in its pathogenicity [10]. This is supported by studies showing cyanide to be the mediating factor in the paralytic killing model of *Caenorhabditis elegans* by *P. aeruginosa* [11]. Cyanide is highly toxic, rapidly diffusing through tissues to irreversibly bind to the terminal oxidases of aerobic respiratory chains inhibiting aerobic respiration. *P. aeruginosa* seems to avoid the toxic effects of cyanide by active detoxification mechanisms and synthesis of a respiratory chain at the end of which is found a terminal oxidase insensitive to cyanide [12–15].

P. aeruginosa is one of many organisms which employ quorum sensing, a process by which extracellular molecules are used to regulate phenotypes in response to population density. In *P. aeruginosa*, cyanide production is regulated, in part, by quorum sensing, with high population densities inducing cyanide synthesis [16–18]. The high *P. aeruginosa* population density of CF mucus maximises quorum-sensing cyanide production and together with its low O_2 concentration and ideal temperature, provides the perfect environmental parameters for *P. aeruginosa* cyanogenesis. The high concentrations of cyanide identified in the sputum and breath of CF patients [5–7] may well affect lung cellular function within human lungs, contributing to the long-term damage known to result from chronic *P. aeruginosa* infection. It may also assist *P. aeruginosa* in excluding other lung pathogens leading to it being the dominant bacterium.

Although high *P. aeruginosa* population densities upregulate cyanide production by quorum sensing, a study measuring the cyanide levels in the sputum of CF and non-CF patients with bronchiectasis showed cyanide levels to be independent of the *P. aeruginosa* bacterial load [11]. This and the absence of cyanide from a small number of sputum/breath samples from *P. aeruginosa* infected patients [5–7] suggest that cyanide production is dependent on the presence of specific *P. aeruginosa* strains as well as the total *P. aeruginosa* load. This theory that cyanide production varies between *P. aeruginosa* genotypes is supported by the finding that the Liverpool epidemic *P. aeruginosa* strain overproduces certain quorum sensing regulated exoproducts such as LasA protease and elastase [19]. To our knowledge, no study has yet assessed the variation in cyanide production by different *P. aeruginosa* genotypes.

It must be considered that cyanide is found in sputum and breath of patients due to cyanogenesis from nonmicrobial sources. Human leukocytes challenged with *Staphylococcus epidermidis* have been reported to produce HCN *in vitro* [20–22]

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and it is possible that the airway inflammation caused by *P. aeruginosa* infection results in cyanide production by leukocytes. If this was correct, however, any organism in the lower respiratory tract causing widespread airway inflammation would lead to cyanide production and yet, cyanide has been shown to be absent from the sputum of CF and non-CF bronchiectasis patients infected with organisms other than *P. aeruginosa* [5, 6].

A recent review of bacterial cyanogenesis in the CF lung [23] concluded that "the detection of cyanide and metabolites from individuals infected with *P. aeruginosa* opens the door to improved monitoring of targeted anti-pseudomonal interventions and possibility to screening for early infection and impending exacerbations using sensitive methodologies to examine exhaled breath."

In the current issue of the *European Respiratory Journal*, STUTZ *et al.* [24] have once again shown that cyanide is increased in relation to *P. aeruginosa* infection in children with CF. However, they also found increased levels of cyanide in children apparently uninfected with the organism and their results suggest that the differences between the two groups of patients were too small to be of value in the individual patient to use cyanide as a marker for the early detection/presence of *P. aeruginosa*. The authors go on to suggest there was a correlation between the level of neutrophils in the sputum samples and the level of cyanide and postulate that cyanide in their samples may be derived from the neutrophils rather than *P. aeruginosa* alone. It is known that cyanide is very water soluble and it is possible that due to their methodology, losses occurred between collection of sputum and measurement of cyanide levels. Such losses are also very dependent on the pH of the specimen. An interesting discussion point is what is the best methodology for accurate and meaningful detection of cyanide/HCN. We know there are differences in results obtained looking at exhaled breath condensate rather than exhaled breath [25].

Whatever, the results are very interesting and give us further information about the intriguing role that cyanide plays in relation to *P. aeruginosa* infection. It is clear that further studies are needed using novel sensitive methodologies to progress our understanding and answer once and for all whether cyanide/HCN measurement does have a role in early identification of the presence of *P. aeruginosa* and whether it can be helpful in future targeted therapies.

STATEMENT OF INTEREST

None declared.

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