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A study to ascertain the potential inhalation hazard of toenail dust

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ABSTRACT

The use of the podiatric nail drill has become an acceptable way of reducing onychogryphotic nails in the clinical setting. The present study has demonstrated the presence of particles that are potentially dangerous to the human lung when they are contaminated with bacterial endotoxin (bacterial cell wall components that may cause an inflammatory response).

Toenail dust collected from podiatric nail drills was examined for size, endotoxin content and the ability to stimulate release of IL-8 (IL- interleukins stimulate the release of other mediators in septic shock and inflammation) from macrophages and lung epithelial cells in vitro. The size distribution revealed a large number of particles that would deposit in the nose, airways and lung alveoli. Endotoxin was detected in extracts of nail particles and, interestingly, a component of these particles was able to stimulate substantial release of IL-8 from lung epithelial cells. Suspensions of toenail particles stimulated IL-8 release from monocyte-derived macrophages. Destruction of the endotoxin with the antibiotic polymyxin B still resulted in IL-8 release, suggesting that the particles themselves initiated the response and not necessarily the endotoxin.

The authors conclude that podiatrists who routinely carry out nail reduction could be inhaling particles that could deposit throughout the respiratory tract, where they could contribute to inflammation by stimulating release of IL-8 from cells via the particles themselves and via endotoxin.

INTRODUCTION

Nail dust

It is generally accepted to be part of a normal working day for podiatrists to use a nail drill, but most practitioners are unaware of how potentially dangerous the dust may be. One study stated that most nail drills have dust extraction facilities attached, but not all the dust created will be extracted away from the practitioner or patient.[1] The study also showed that larger nail dust particles fall to the floor, whereas the smaller airborne particles that have not been extracted could easily be inhaled. Another study compared two types of dust extractor, vacuum and water spray.[2] These systems were tested to evaluate how dust was 'expelled' into the environment, and their effectiveness was found to be 24% and 91.6%, respectively. This would suggest that although the extractors do reduce the amount of dust in the immediate area, there is a huge variation in the effectiveness of the systems. A study to ascertain the efficiency of nail dust extraction systems was undertaken, with levels of 12–53% of dust being 'lost' to the atmosphere.[3]

It is therefore important to ascertain the effects of inhalation of fungal nail dust on the podiatrist, and how differing sizes of dust particle may irritate different areas such as the nasal area, upper respiratory tract and the alveoli of the respiratory system.[3,4] Particles that can be inhaled into the respiratory tract can be categorised into three types. Firstly, inhalable particles that deposit in the mouth, larynx and pharynx and are larger than 20µm in diameter; secondly, thoracic that deposit beyond the larynx but above the airways and are between 5-20µm in diameter; and, thirdly, respirable, which can deposit 'deeper' in the bronchioles and the alveolar ducts. These smaller particles are 5µm and less in diameter and thought to be the most irritant and dangerous, causing emphysema and fibrosis.[4]

Analysis of dust particles using electron microscopy and topography allows the characteristics of each type of particle, e.g. size and attached bacterial/fungi, to be identified. A study found that this type of characterisation demonstrates that nail dust consists of keratin plates, keratin hydrolysates, fungi, fungal arthrospores and filaments.[3] The study also concluded that nail dust ranged from 0.1µm to 100µm in diameter, which could result in 31% of nail dust being deposited in the lungs. In addition, 86% of the dust was sized between 0.8µm and 1.6µm, which means that potentially 16% of dust inhaled in a cloud of nail dust may be deposited in the alveoli.[3] A study comparing two groups of podiatrists demonstrated that antibody levels of IgE were raised in 31% of qualified podiatrists but not in the student podiatrist control group.[2] This would, therefore, suggest that this type of sensitisation becomes more frequent in podiatrists the longer they have been working and been exposed to nail dust.

A questionnaire survey of respiratory problems in podiatrists showed that 49% of the sample were affected by nail dust, with nasal problems, eye irritation or breathing problems.[5] Immunological assays performed in the same study demonstrated that a percentage of the podiatrists had precipitating antibodies to

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Trichophyton rubrum, with the immune response increasing with exposure time.[5] If nail dust cannot be stopped from entering the body by the body's natural first-line defences (e.g. nasal hair, mucous), an immune hypersensitivity reaction will often occur.[6] Another study utilising questionnaires, found that 47.6% of State Registered Chiropodists had throat irritation, 28.6% had experienced chest tightness, 20.8% experienced wheezing, 14.3% were short of breath and 12.9% demonstrated abnormal lung function.[7]

Contamination of dust particles

It is thought that endotoxin may contaminate most, if not all, organic dusts,[8] it is also known that endotoxin found in wool particles causes acute inflammation in rat lung and is toxic in human lung.[5] Endotoxin is everywhere in the environment, but is normally only found entering the lung on contaminated dust, such as nail dust. Endotoxins are lipopolysaccharide (LPS) structures, which are found in the outer membranes of Gram-negative bacteria , such as *E. coli, Salmonella sp.* and *Pseudomonas sp.*[9] Endotoxins are heat and chemically stable to normal podiatric disinfectants and need to be boiled for over 30 minutes (autoclaved) before breaking down and being destroyed.

Inflammation in the lung

When lung disease or lung inflammation occurs, this is usually due to failure of the body's physical natural defence mechanisms. Particles can cause direct physical damage to the lung tissue or may go on to activate the complement system, which then results in inflammation. Complement is involved in the destruction of bacteria and other organisms. The complement cascade is devised from around 20 serum glycoproteins, which undergo cleavage in one of either two pathways. Antigen and IgM activate pathways, whereas IgA and endotoxin, amongst other things, activate the alternative pathway.[6] Cytokines are soluble messenger molecules that trigger a specific type of cell and signal the cell to divide or to become active at a certain area of the body.[6] Interleukins (ILs) are specific to white cells and are important as they are secreted by macrophages and epithelial cells, and promote inflammation by attracting neutrophils and basophils to the site of bacterial or endotoxin invasion.

The present study was undertaken to provide an understanding of the size, shape and affects if nail dust particles on lung epithelial cells.

MATERIALS AND METHODS

Dust sample collection

Samples of nail dust were obtained from three podiatry drill extraction bags, under a hood (to avoid any inhalation of the dust). The bags had previously not been changed for a minimum of one week and a maximum of two weeks. The three samples were mixed together to allow particles from each nail drill to be equally dispersed in the final sample.

Microscopy

Nail dust samples were suspended in sterile saline to a final concentration of 1mg/ml, and sonicated for five minutes. On analysis this proved to be the optimal concentration, allowing the particles to be viewed easily and sized, using light, transmission and scanning microscopy.

Endotoxin estimation

One hundred microlitres of the previously prepared leachates were assayed for endotoxin using an endotoxin assay kit (Associates of Cape Cod, Liverpool).

IL-8 release

A549 lung epithelial cells were grown in continuous culture and plated into wells of a 24-well plate. The plate was incubated for 24 hours at 37 degrees C and then an equal volume of TGP (Toenail ground particles) leachate was added to each well. Plates were incubated for 6 and 24 hours and the supernatant removed and stored at 80 degrees C until further analysis for IL-8 by conventional ELISA (Enzyme-linked immunosorbent assay). Human monocytes were isolated from fresh peripheral blood and maintained in culture for five days until they attained the morphology of macrophages. These macrophages were exposed to various concentrations of (a). TGP and (b). TGP that had been treated with polymyxin B (to destroy the endotoxin). The levels of IL-8 were then measured by conventional ELISA.

Ethical approval was sought and granted by Queen Margaret University College, Napier University and ELEGI Colt Laboratories, University of Edinburgh.

RESULTS

Size distribution of nail particles

The majority of the particles were found to be less than 10µm in size, indicating that most of them were respirable. None of the particles was greater than 71µm, although when analysing the particles it appeared that some particles were aggregated (Figure 1).

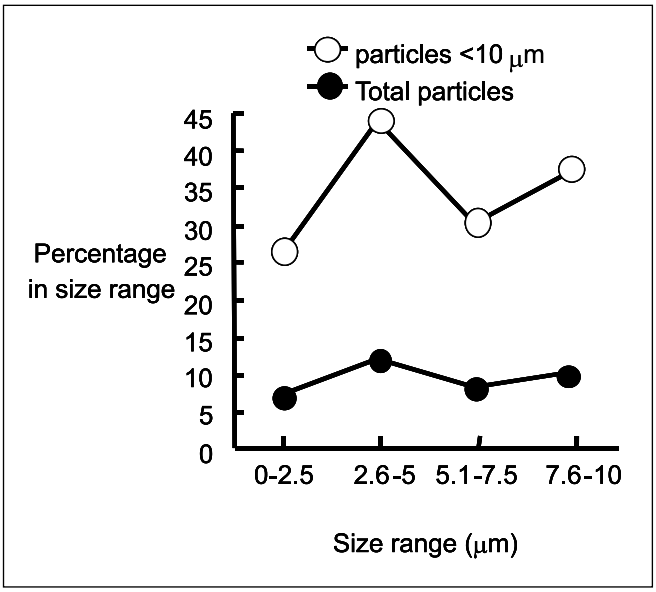

Figure 1. Size distribution of TGP obtained from 100 randomly selected particles by high power light microscopy. Most particles are less than 10µm in size.

Morphology of nail particles

The images obtained using transmission electron microscopy (TEM) and scanning electron microscope (SEM) showed a fairly uniform shape of particle. The particles were plate-shaped with ragged edges in most cases (Figure 2), and most of were flat-shaped. The optical density was lower in the lighter areas, indicating that the particle was thinner in such areas.

Endotoxin content of nail particles

The endotoxin content of the leachates of the nail dust is shown in Figure 3. In three separate samples, the levels of endotoxin released from 1mg into 1ml of saline were 8–10 EU (expirable units) per mg of TGP.

IL-8 release

The ability of leachate from TGP to stimulate IL-8 release from A549 lung epithelial cells is shown in Figure 4. There was substantial stimulation of IL-8, up to 100pg/ml from a control level of 60pg/ml. Macrophages released similar amounts of IL-8 at the highest concentration of TGP. There was little effect of polymyxin B treatment in affecting the IL-8 response, suggesting that endotoxin had little role to play and that the particles themselves were important (Figure 5).

DISCUSSION

The present study was initiated to determine the potential for toenail grinding particles, collected from reducing nails, to cause inflammation of lung tissue. A number of previous reports have indicated possible adverse health effects from TGP.[3,4,7,10-12]

Characterisation of the particles of nail dust, by light microscopy, showed that they are generally uniform in both shape and size, ranging from 0.1µm to 71µm, most within the range 0–10µm and the majority of these 2.6–5µm in size. This would suggest that most of the particles in the sample were respirable and would therefore be able to enter the lung. The images from the SEM

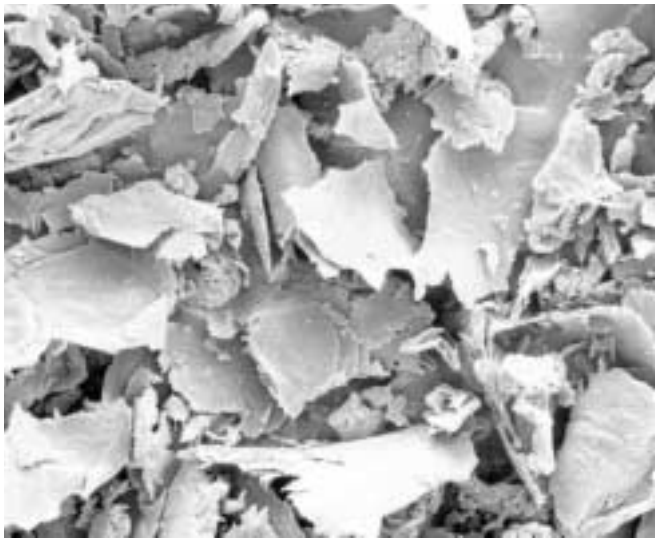

Figure 2. Scanning electron micrograph of TGP. Note large plate-like particles and smaller compact particles.

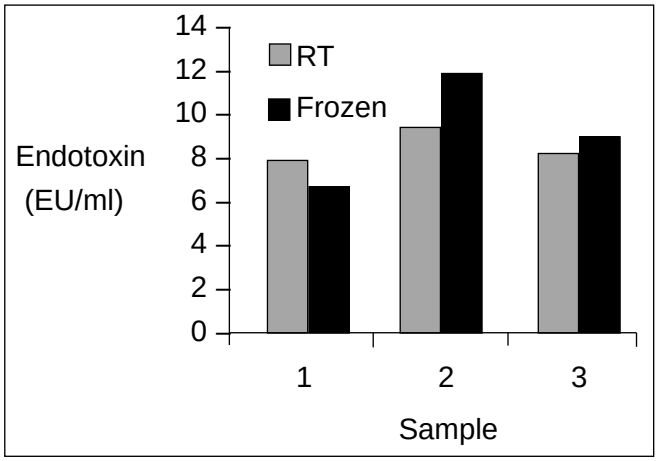

Figure 3. Endotoxin level in 1mg/ml extract of TGP. RT = retained at room temperature for three weeks prior to measurement; Frozen = stored frozen for three weeks prior to measurement.

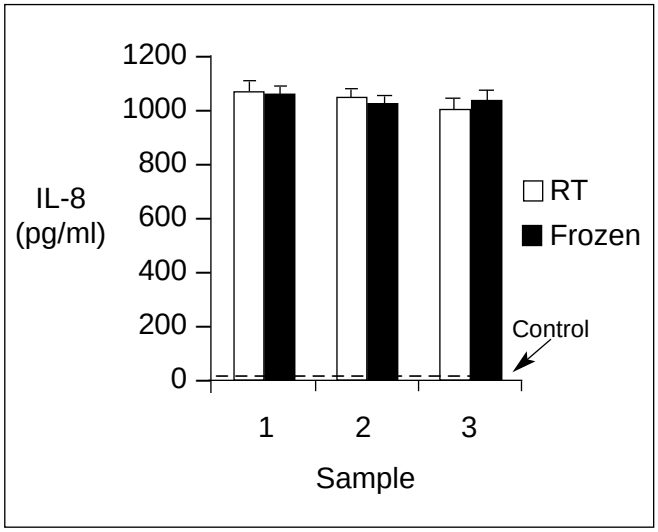

Figure 4. IL-8 release by A549 epithelial lung cells exposed to leachate from TGP (bar denotes standard deviation).

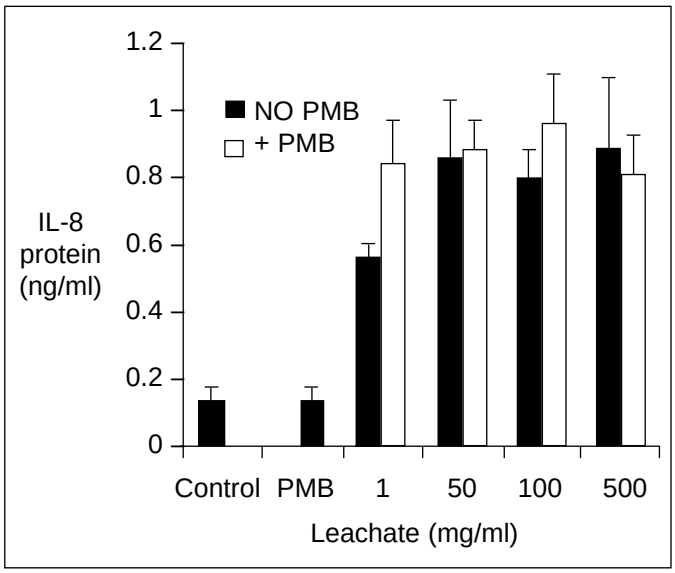

Figure 5. IL-8 release from human monocyte-derived macrophages treated with TGP suspensions either untreated or treated with polymyxin B to remove endotoxin (bar denotes standard deviation).

showed that most of the particles were plate-shaped, giving them unique aerodynamic properties. Rather than dropping to the ground like a round-shaped particle would, the plate-shaped particle would possibly fall in the same fashion as a piece of paper with a side-to-side oscillation. This suggests that the particles may be airborne for a longer period of time, and as a result there could be an increased chance of the particle being inhaled by podiatrists.

It was found that TGP collected from nail drills was endotoxin-rich and able to stimulate large-scale release of the chemokine IL-8 from target cells, and so could exacerbate an existing lung condition such as asthma or have a chronic inflammatory effect in the lung. Endotoxin was readily measurable on the TGP, and storing TGP at room temperature did not increase the levels of endotoxin over storing it frozen.

The presence of endotoxin on nail particles places them in a range of organic dust that have their adverse effects through the well-documented cell-stimulatory effects of endotoxin. These include PM10, i.e. particles less than 10 microns[13] and wool dust and grain.[5]

The macrophage technique utilised in the present study, showed no effect with the addition of polymyxin B, demonstrating that the particles themselves may be important in addition to the effects of endotoxin. IL-8 is a potent neutrophil-attracting chemokine.[14] Increased production of IL-8, or the rat homologue MIP-2, by macrophages and epithelial cells in response to dust particles has been implicated in the pro-inflammatory stage of a range of different particles such as quartz[15] and PM10.[16]

Interpreting these data to potential health risks in podiatrists

In order to interpret properly the data on TGP that is presented here, it is necessary to consider the relationship between particle size, site of deposition in the lungs and inflammatory lung diseases. This is necessarily a simplified exposition of a complex subject that can be found in more depth elsewhere.[17] The site at which particles deposit within the lungs will receive the 'dose' of particles, and, if the particles are harmful, then the injury and finally the pathology will arise in this site. The anatomical compartments are shown in diagrammatic form in Figure 6, being the nasopharyngeal region, the airways (or bronchi) and the alveolar region. Table 1 shows examples of the types of response that are found in the various compartments; thus asthma is a disease of airways and emphysema is a disease of the alveolar region.

Table 2 illustrates the relationship between particle size and site of deposition. It illustrates the important point that the upper airways of the lung act as a filter, or more correctly an elutriator, in that particles are sorted by sizes. As air passes deeper into the lungs the larger particles deposit, leaving gradually smaller particles to penetrate to the deeper parts of the lung, the alveolar region. Subsequently, whilst large particles (in the order of 100mm) can gain access to and deposit in the nasal and pharyngeal regions, only very small particles (less than 10mm) ever reach the alveoli. The particles that cause diseases of the alveolar region, such as emphysema and fibrosis (interstitial lung disease), are therefore only those in this small size range. By the same argument the particles that cause asthma can be in the intermediate and small size range, while particles of all sizes can cause hay fever.

The relevance of this to the present study is that the toenail dust particles were in all size ranges and so have the potential to cause pro-inflammatory effects throughout the lungs of podiatrists. They could, in theory at least, contribute to all of the diseases shown in Table 1.

Asthma is a highly prevalent disease, which will afflict a number of podiatrists. While the effects described in the present study are not directly related to asthma, which is a special case of inflammation resulting from an overly exuberant and inappropriate immune response to antigen in some individuals, the results demonstrate a general ability of toenail particles to stimulate the airway lining epithelial cells and macrophages to release pro-inflammatory mediators. This could result in inflammation in the absence of asthma, but could also exacerbate asthmatic airways that are 'primed' by chronic inflammation.[11]

Smoking and smoking-related lung disease (Chronic Obstructive Pulmonary Disease) are also common and will affect a proportion of podiatrists. COPD patients have inflamed airways[18] and these may also be susceptible to worsening of the inflammation

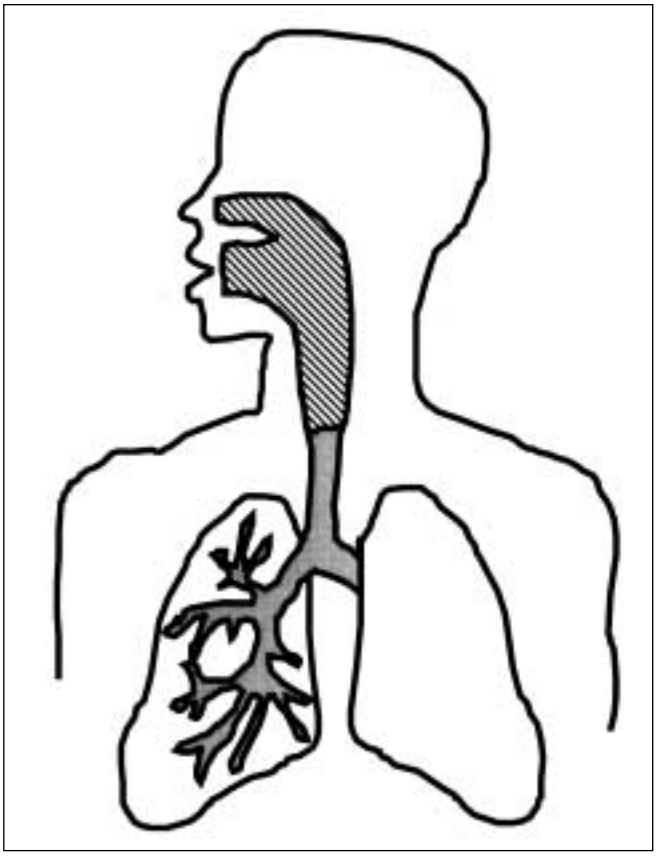

Figure 6. Diagrammatic representation of the lunds and anatomical compartments.

	SIZE		
Compartment	**100µm**	**10µm**	**1µm**
Nasopharynx	yes	yes	yes
Airways	no	yes	yes
Alveoli	no	no	yes

Table 1. The lung compartments in which particles of various sizes would be expected to deposit.

Compartment	Disease
Nasopharynx	Hay fever
Airways	Asthma/bronchitis
Alveoli	Fibrosis/Emphysema

Table 2. Differing pathological responses arising in anatomical lung compartments.

caused by dust particles including toenail dust particles. The smaller particles could penetrate to the alveolar regions and stimulate inflammation in the delicate alveolar tissues, resulting in fibrosis or emphysematous change. Emphysema, or destruction of alveoli, causes airway narrowing and is part of the COPD syndrome, and so could be worsened by toenail dust exposure in podiatrists who smoke. Fibrosis causes a number of effects on lung elasticity as well as gaseous exchange and needs to be specifically investigated in podiatrists. The risks of ocular infection by nail dust have also been highlighted.[12]

CONCLUSION

The authors conclude that the toenail dust generated from a podiatric nail drill is of a highly respirable fraction because of the size and shape of the particles. The majority of the particles measured in this study are capable of depositing in the alveoli and potentially initiating an inflammatory response. The sample showed an extremely high content of endotoxin, which has the potential to cause an immune response with IL-8 release from lung A549 epithelial cells, and there therefore has the potential to cause the same response in normal pulmonary epithelial cells, i.e. *in vivo*. Reducing toenails with a drill on a regular basis will expose podiatrists to some risk, especially those with airway disease such as asthma.

The safe use of nail drills, extraction and filters requires that they are routinely serviced and maintained to ensure their efficiency and thus protect the podiatrist. Practitioners are recommended to read the Society of Podiatrists and Chiropodists Guidelines 2000, on the use of podiatric nail drills.

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