

Toenail Dust Particles: a Potential Inhalation Hazard to Podiatrists?

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Toenail dust collected from podiatrist's nail drills was examined for size, endotoxin content and ability to stimulate release of interleukin 8 (IL-8) from macrophages and lung epithelial cells *in vitro*. The size distribution revealed a large number of particles in the size ranges that would deposit in the nose, airways and lung periphery. Endotoxin was readily measurable in aqueous extracts of the nail particles and a soluble component of the nail dust particles was able to stimulate substantial release of IL-8 from epithelial cells. Suspensions of toenail particles were tested for ability to stimulate IL-8 release from monocyte-derived macrophages; treatment with polymyxin B to remove endotoxin had no effect. We conclude that podiatrists who routinely carry out nail drilling could be inhaling particles that can 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.

Keywords: endotoxin; IL-8; toenail dust

INTRODUCTION

The reduction of overgrown nails by drilling is a common procedure used by podiatrists. This operation necessarily generates finely divided nail particles that become airborne (Abramson and Wilton, 1985b). Although most nail drills now have dust extraction facilities attached, not all the dust created will be trapped because of a number of factors, including poor maintenance, bad set-up, etc. Toenail grinding dust (TGD) consisted of keratin, keratin hydrolysates, fungi, fungal arthrospores and filaments and other microbial debris (Abramson and Wilton, 1985a). Immunoglobulin E levels were raised in 31% of podiatrists, but there was no increase in the student control group used in the study (Davies, 1984), and 49% of state registered chiropodists who returned a questionnaire stated that nail dust caused nasal or eye irritation or breathing problems (Davies *et al.*, 1983); the same study reported that a percentage of these podiatrists had precipitating antibodies to the fungus *Trichophyton rubrum*.

MATERIALS AND METHODS

Collection of TGD

Samples of TGD were taken from the collection bag of three Suda nail drills from the podiatric clinic in Inchkeith House Clinic, Edinburgh. The bags had not been changed for between 1 and 2 weeks. The three samples were mixed together to allow particles from each nail drill to be equally dispersed in the final sample. This sample was then halved; one half was placed in a freezer at –80°C and the other was kept at room temperature. These samples were left in this state for 3 weeks.

Particle size

A dilute suspension of TGD was prepared and 100 random particles were sized using a high power light microscope and an eyepiece graticule and the size distribution calculated.

Endotoxin measurement

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

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IL-8 release

A549 epithelial cells were grown in continuous culture and plated into wells of a 24-well plate. The plate was incubated for 24 h at 37°C and then an equal volume of TGD leachate was added to each well. Plates were incubated for 6 and 24 h and the supernatant removed and stored at -80°C until further analysis for interleukin 8 (IL-8) by conventional ELISA. Human monocytes were isolated from fresh peripheral blood and maintained in culture for 5 days until they attained the morphology of macrophages. These were exposed to various concentrations of TGD particles and TGD that had been treated with polymyxin B (PMB) to remove endotoxin. The levels of IL-8 were measured by conventional ELISA.

RESULTS

Size distribution

The size distribution of 100 randomly selected TGD particles was as shown in Fig. 1. Approximately one-third of all particles were <10 µm.

Morphology

Figure 2 shows a scanning electron microscope image of the TGD. It reveals that the particles range in size up to ~40 µm, although it also shows the presence of many smaller particles. The larger particles are clearly flake- or plate-like in shape, whilst the smaller particles are generally compact.

Endotoxin

The endotoxin contents of leachates of the TGD are shown in Fig. 3. In three separate samples the levels of endotoxin released from 1 mg into 1 ml of saline were 8–10 EU/mg TGD.

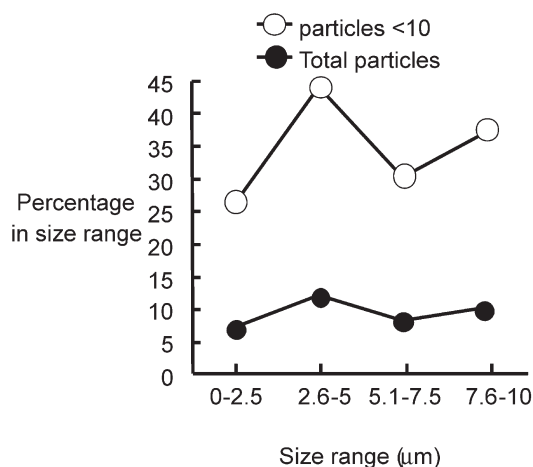


Fig. 1. Size distribution of TGD as obtained from 100 randomly selected particles by high power light microscopy.

Stimulation of IL-8

The ability of the leachate from TGD to stimulate release of IL-8 from A549 epithelial cells is shown in Fig. 4. There was substantial stimulation of IL-8 release, up to ~1000 pg/ml from a control level of 60 pg/ml. Macrophages released similar amounts of IL-8 at the highest dose of TGD. There was little effect of PMB treatment on the IL-8 response, suggesting that endotoxin had little role to play and that the particles themselves were important.

DISCUSSION

We set out to determine whether there was a potential for TGD, collected from podiatric grinding of toenails, to cause lung inflammation. A number of previous reports have indicated possible adverse health effects of TGD (Davies *et al.*, 1983; Abramson and Wilton, 1985a,b; Gatley, 1991; Ward, 1995).

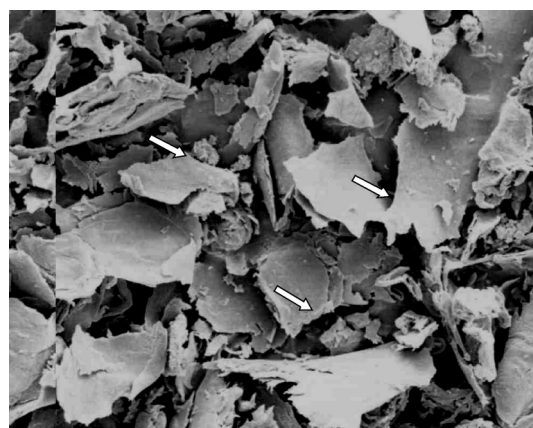


Fig. 2. Scanning electron micrograph of TGD. Note large plate-like particles and smaller compact particles. Original magnification $\times 40000$.

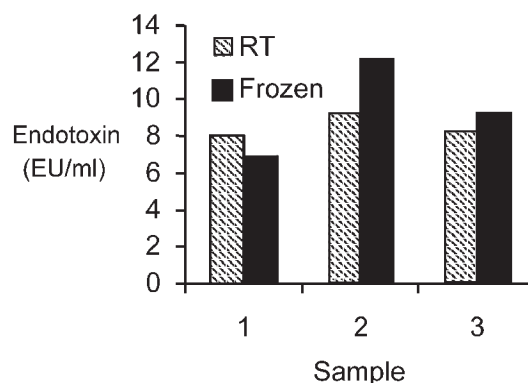


Fig. 3. Endotoxin level in 1 mg/ml extract of TGD. RT, retained at room temperature for 3 weeks prior to measurement; Frozen, stored frozen for 3 weeks prior to measurement.

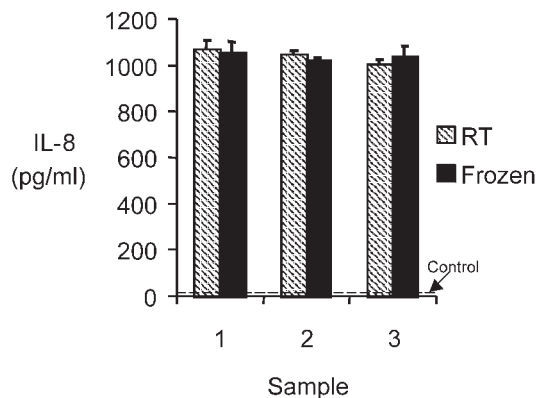


Fig. 4. IL-8 release by A549 epithelial cells exposed to leachate from TGD.

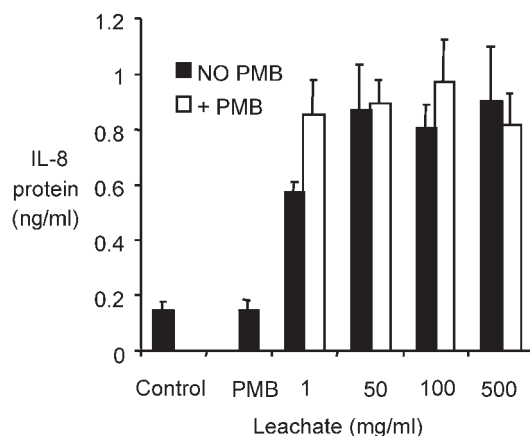


Fig. 5. IL-8 release from human monocyte-derived macrophages treated with TGD suspensions either untreated or treated with PMB to remove endotoxin.

We found that TGD collected from the collection bag of nail drills was highly respirable, endotoxin-rich and able to stimulate large scale release of the chemokine IL-8 from relevant target cells. This suggests that podiatrists who are regularly carrying out this procedure could be at risk from inhalation of this dust, which could exacerbate existing lung conditions such as asthma, or have a chronic inflammatory effect in the parenchyma.

The size distribution of the TGD included significant proportions of particles that could deposit in the upper respiratory tract, the airways and the respiratory zone (Schlesinger *et al.*, 1998). The flake-like shape of the particles could affect deposition since plate-like particles will remain airborne because of their unusual aerodynamic resistance. Deposition throughout the respiratory tract means that the pro-inflammatory effects of TGD could impact on nasal symptoms and airway diseases such as asthma, as well as having effects on the pulmonary parenchyma. Increased levels of PM₁₀ particles, known to stimu-

late IL-8 release from lung cells (Gilmour *et al.*, 2001), are associated with exacerbation of asthma (Pope and Dockery, 1999). Since TGD stimulates IL-8 release, it may also cause a worsening of asthma. This exposure may explain the increased incidence of respiratory tract symptoms in podiatrists (see above).

Endotoxin was readily measurable on the TGD and storage at room temperature did not increase the levels of endotoxin over TGD that was stored frozen. The presence of endotoxin on particles places TGD alongside a range of organic dusts that have adverse effects through the well-documented cell stimulatory effects of endotoxin. These include PM₁₀ (Ning *et al.*, 2000), wool dust and grain dust (Brown and Donaldson, 1996). The macrophage results, showing no effect of PMB treatment of the particles, demonstrate that the particles themselves may also be important, in addition to the effects of endotoxin.

IL-8 is a potent neutrophil-attracting chemokine (Streiter and Kunkel, 1997). 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 activity of a range of different particle types, such as quartz (Driscoll *et al.*, 1997) and PM₁₀ (Gilmour *et al.*, 2001).

In conclusion, this pilot study suggests that the grindings from podiatrist's nail drills have the potential to deposit throughout the respiratory tract and stimulate release of IL-8 from lung cells. Grinding toenails on a regular basis may explain the symptoms described in podiatrists, and podiatrists who regularly grind nails may be at risk from TGD exposure, especially those with airway diseases such as asthma.

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