INTRODUCTION

Endotoxin, a heat-stable toxin associated with Gram-negative bacterial infections, is composed of LPS derived from the bacterial cell wall. Most of the pathogenic effects seen in Gram-negative bacterial infections are mediated by an endotoxin (Natanson et al., 1994); the associated clinical syndrome may even occur in the absence of bacteraemia (Danner et al., 1991; Graham & Brass, 1994). Endotoxin has been implicated in the pathogenesis of hepatotoxicity, hepatorenal failure, hepatic encephalopathy (Shibayama, 1992; Odeh, 1994), periodontitis (Trope et al., 1995), mastitis (Tyler et al., 1994), adult respiratory distress syndrome (Herbert et al., 1992; Graham & Brass, 1994), disseminated intravascular coagulation (Graham & Brass, 1994), humidifier fever (Flaherty et al., 1984; Mamolen et al., 1993) and sick building syndrome (Teeuw et al., 1994).

Dental waterslines contain large numbers of Gram-negative bacteria. Endotoxin, a component of such organisms, has significant health implications. Paired samples of dental unit water and the aerosols generated during dental procedures were collected, and assayed for bacteria and endotoxin levels, using heterotrophic plate counts and the Limulus amoebocyte lysate test. Consistent with published studies, the extent of bacterial contamination in the dental waters sampled for this investigation surpassed the levels associated with potable water, with counts in excess of $2.0 \times 10^6$ c.f.u. ml$^{-1}$ in some samples. Correspondingly high concentrations of endotoxin [up to 15 000 endotoxin units (EU) ml$^{-1}$] were present in the water. A statistically significant Spearman correlation coefficient of $\rho=0.94$ between endotoxin (EU ml$^{-1}$) and bacterial load (c.f.u. ml$^{-1}$) was demonstrated. All of the aerosol samples contained detectable endotoxin. Further studies of the consequences of dental endotoxin exposure, and evaluation of means to prevent exposure, are warranted.
plate counts (APHA, 1985). Samples (2–3 ml) were collected in sterile, pyrogen-free 12 × 75 polystyrene tubes, avoiding any contact between the instrument parts and the tube during collection. Samples were shipped overnight to the laboratory at 4 °C.

Aerosols were collected using AGI-30 glass impingers (Ace Glass) containing endotoxin-free sterile water, as described by Trudeau & Fernandez-Caldas (1994). Impingers were set up at a distance of 60 cm from the area of dental work in a patient’s mouth and a total of 0.33 m³ of air was sampled for each of eight tested operatories: four from the area of dental work in a patient’s mouth and a total of Fernández-Caldas (1994). Impingers were set up at a distance of 60 cm from the area of dental work in a patient’s mouth and a total of 0.33 m³ of air was sampled for each of eight tested operatories: four from the area of dental work in a patient’s mouth and a total of 47 separate water/aerosol pairs for our analyses.

Bacterial contamination. Bacterial contamination of the water samples was assessed by plating 100 µl aliquots of serial dilutions of each sample onto R2A plates and incubating as described previously (Williams et al., 1993).

At each of the aerosol collection sites, additional collections were made using Anderson plate chambers. In this procedure, air is drawn over the surface of agar plates through steel plates, which deflect the particles onto the surface of the R2A agar for subsequent incubation and colony formation (Turner & Hill, 1975). In this way, the number of bacteria may be determined qualitatively, and the resultant colonies permit standard microbiological isolation and identification methods to be applied.

Endotoxin assay. The presence of endotoxin in the samples was assessed using the Limulus amoebocyte lysate (LAL) test (USDHHS, 1987), commercially available as a Pyrotell kit (Associates of Cape Cod). The LAL assay is exquisitely sensitive to endotoxin, and mandates devoted adherence to the use of endotoxin-free glassware and plasticware (including tubes and pipette tips), diluent, etc. The assay was performed in accordance with the specified protocol. Results were obtained in endotoxin units (EU); 10 EU corresponds to 1 ng endotoxin. Commercially available endotoxin standards were used as controls.

Statistical analysis. The Spearman rank correlation coefficient for non-parametric data was employed to analyse the relationship between endotoxin and bacterial contamination levels.

RESULTS AND DISCUSSION

Contamination levels

Consistent with all studies to date, the extent of bacterial contamination in the dental waters sampled for this investigation far surpassed the levels associated with potable water, with counts in excess of 2.0 × 10⁶ c.f.u. ml⁻¹ in some samples. Correspondingly, high concentrations of endotoxin (up to 15 000 EU ml⁻¹) were present.

The relationship between bacterial concentrations and the results of the endotoxin assay are shown in Fig. 1. A Spearman rank correlation coefficient of ρ=0.94 between EU ml⁻¹ and c.f.u. ml⁻¹ was demonstrated, which was significant at P<0.05.

Aerosol sample cultures produced a varied mixture of bacterial organisms, which were rapidly overgrown by the abundant growth of a number of fungal varieties. This precluded quantification of bacterial numbers in aerosols. Nevertheless, a variety of bacteria was isolated, including Gram-positive cocci, Gram-positive cocccobacilli, Gram-negative cocci and Gram-negative bacilli. Not all of the isolates were identifiable using standard microbiological and biochemical profiling techniques, but of those that could be definitively assigned to species, the following were found: Staphylococcus epidermidis, Staphylococcus aureus, Staphylococcus haemolyticus, Micrococcus spp., Micrococcus varians roseus, Pseudomonas vesicularis, Sphingomonas paucimobilis, Acinetobacter spp. and Flavomonas spp.

All of the aerosol samples collected in impingers contained detectable endotoxin, although the concentration did not exceed 2.7 EU m⁻³ in any instance. No endotoxin was detectable in control air samples.

Water obtained from properly functioning distillation, reverse osmosis and ultrafiltration systems generally has undetectable levels of endotoxin. Clearly, the concentrations seen in the dental water samples far exceeded any acceptable standard for water intended for medical purposes. The generally acknowledged range of 0.06–0.5 EU ml⁻¹ for medical devices (including liquid devices), depending on application, applies to irrigation fluids and is regulated by the US federal government (USDHHS, 1993).

We found levels of endotoxin in dental water several orders of magnitude below that reported in two published reports,
both from the same investigator (Szymanska 2005a, b). Compared with others (Putnins et al., 2001; Fulford et al., 2004), our water endotoxin levels exhibited a broader range of values, encompassing their results but extending to both higher and lower levels. The reason for the discrepancy with the former (Szymanska 2005a, b) is not clear. Such a large difference in endotoxin level cannot be attributable solely to microbial content of the waterlines, as c.f.u. counts between those studies and ours were comparable. It is possible that significantly higher levels of endotoxin existed in the municipal water supplying the operatory and were possible that significantly higher levels of endotoxin existed solely to microbial content of the waterlines, as c.f.u. counts higher and lower levels. The reason for the discrepancy of values, encompassing their results but extending to both 2004), our water endotoxin levels exhibited a broader range between the levels was sought as a 'simple test for contamination is likely to be the Gram-negative organisms in the proximate source of the dental water endotoxin reading the endotoxin levels as EU rather than μg would bring these values into close agreement with that seen in this study, and those of Fulford et al. (2004) and Putnins et al. (2001). Aerosolized endotoxin levels documented in the present study were also exponentially lower than those found by Szymanska (2005a), who also found that disinfection of the dental unit waterlines, whilst decreasing the level of endotoxin detectable in the water, had no significant effect on the aerosolized endotoxin levels.

In light of the significant correlation demonstrated in Fig. 1, the proximate source of the dental water endotoxin contamination is likely to be the Gram-negative organisms resident in the dental waterline biofilms (Mayo et al., 1990; Pankhurst & Philpott-Howard, 1993; Williams et al., 1993). The correlation between bacterial and endotoxin contamination levels observed in this study is at odds with the findings of others (Bourassa et al., 1995; Fulford et al., 2004; Szymanska, 2005b). In those studies, a correlation between the levels was sought as a 'simple test for monitoring bacterial contamination'. They found no significant relationship between c.f.u. and endotoxin concentrations, even when the c.f.u. varied by up to three orders of magnitude. One group (Bourassa et al., 1995) proposed that this lack of relationship may be due to bacterially produced inhibitors of the Limulus chromogenic test employed in their investigation (Roth et al., 1990). The LAL assay may, in fact, be less prone than the chromogenic test to such inhibitors. However, another investigator failed to demonstrate a significant correlation using the same LAL techniques employed in our laboratory (Szymanska, 2005b), although the possibility of exogenous endotoxin being supplied in the municipal water could confound this, as noted above. The range of both bacterial and endotoxin levels found in our study was broader than that seen by Fulford et al. (2004), and could explain why a statistically significant correlation was not found in the latter. Other explanations could relate to sample size, variation in aliquot-handling technique or contamination of diluents or laboratory-ware by exogenous endotoxins. Whatever the cause of the differences, whilst there is not an adequate basis on which to compute the number of bacteria ml⁻¹, the correlation between endotoxin and c.f.u. ml⁻¹ is clear.

**Implications**

The presence of endotoxin in dental water has potential clinical significance, both medically and dentally. Endotoxin stimulates the production of numerous cytokines, which resulting in tissue injury (Graham & Brass, 1994). These may inhibit healing following dental or periodontal treatment. The significance of endotoxin in the pathogenesis of periodontitis is well documented (Trope et al., 1995), and irrigation of highly vascular mucosal lesions with endotoxin-laden water during treatment for this condition is, at the very least, medically inappropriate. Irrigation of the site of any dental intervention with endotoxin-laden water has the potential for introduction of the endotoxin into the patient’s bloodstream. Experimental injection of endotoxin into the systemic circulation of healthy volunteers elicits the signs and symptoms of endotoxaemia, including fever, elevated white blood cell count, elevated blood concentrations of stress hormones and decreased blood oxygenation (Suffredini et al., 1989; van Deventer et al., 1990; Herbert et al., 1992). Endotoxaemia is typically associated with infection, but significant exposure via inhalation (Castellan et al., 1987; Teeuw et al., 1994) has been reported. Little is known about the significance of aspiration, ingestion, mucosal or dermal exposure.

Of potentially greater significance than the occasional endotoxin exposure of patients undergoing dental care is the continuous exposure of those involved in the dental and dental-hygiene professions. Chronic endotoxin inhalation represents an occupational hazard to these groups. In other settings, Gram-negative organisms in the biofilms of plumbing and climate-control ductwork contribute medically significant quantities of endotoxin to their surroundings (Costerton et al., 1987; Hugenholtz & Fuerst, 1992; Szymanska, 2005b). Inhaled endotoxin significantly lowers spirometric values in otherwise healthy subjects (Castellan et al., 1987; Herbert et al., 1992). Breathing endotoxin-tainted air has serious implications for exacerbation of chronic obstructive pulmonary disease and asthma in individuals with pre-existing conditions (Michel et al., 1996). Studies linking airborne endotoxin to sick building syndrome (Teeuw et al., 1994) suggest that even uncompromised individuals are at risk for the development of medical conditions due to inhaled endotoxin. Inhaled endotoxin has been suggested as the source of occupationally acquired asthma in dentists (Pankhurst et al., 2005). Whilst there is no specific concentration of airborne endotoxin above which is defined as hazardous, experimental animals show respiratory dysfunction at 0.3 μg m⁻³. The use of face masks to prevent exposure is marginal at best, with inconsistent
effects that are heavily dependent on aerosol droplet size and a host of other variables (Lipp, 2003).

In addition to the clinical implications of dental water endotoxin, there are medico-legal ramifications. On top of the potential liability incurred by exposing patients to endotoxin, there is risk from an employee health perspective. In the USA, the Indoor Clean Air Act Amendments of 1990 mandate that, within buildings, exposure to airborne pollutants cannot exceed levels of exposure in the local outside air: dental office airborne endotoxin exposure may cross that threshold. The European Commission similarly regulates indoor air quality in the workplace (Stevens & Palmigianob, 1995). A World Health Organization working group has issued statements delineating the right to healthy indoor air, statements that have been successfully cited in lawsuits (Molhave & Krzyzanowski, 2003). Blaming an exacerbation of chronic obstructive pulmonary disease or asthma on workplace exposures could lead to both civil and criminal penalties.

The endotoxin literature contains an interesting report on the inhibition of Legionella growth within endotoxin-treated macrophages (Egawa et al., 1992). In this study, macrophages from a murine strain permissive for Legionella growth became highly resistant to growth of the organism when pre-treated with endotoxin. This enhanced cytolysis activity occurs at some point subsequent to the initial bacteria–macrophage interaction. It is tempting to speculate that the high levels of endotoxin delivered in dental water might exert some sort of protective effect, contributing to the infrequency of severe legionellosis in those exposed (Fotos et al., 1985; Reinhalter et al., 1988) to the high levels of Legionella present in dental water (Atlas et al., 1995).

Efforts to control the dental-line biofilm, the source of endotoxin in the dental operatory, have been largely disappointing. flushing, long advocated as a solution, has been shown to be of no benefit (Santiago et al., 1994), and biofilms are resistant to chemical disinfection (Mills & Karpay, 2002). Filtration is effective at removing the planktonic bacteria from the dental water (Mayo & Brown, 1999), but has no effect on the core biofilm and its endotoxin production.

In conclusion, this study has demonstrated that dental unit water contains high concentrations of endotoxin, and that there is a statistically significant positive correlation between endotoxin and the bacterial load present. This water is readily aerosolized during routine dental work. Exposure to either the endotoxin-laden water or the aerosolized endotoxin represents a potential health threat. Further epidemiological and clinical studies of the consequences of dental endotoxin exposure, and the means by which this exposure may be prevented, are warranted.

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REFERENCES


