THE EFFECT OF RADIO-OPACIFIERS IN BONE CEMENT ON AN IN VIVO MODEL OF ASEPTIC LOOSENING.

INTRODUCTION

Aseptic loosening is the commonest cause of failure of total joint replacements. The pathogenesis of this process remains controversial but appears to involve prosthetic migration, joint fluid pressure and the host response to particulate debris. The Swedish Hip Registry has shown that the type of bone cement used affects the risk of revision for aseptic loosening. It has been suggested that this effect is mediated by the host response to the type of radio-opaque agent contained within bone cement particulate debris, as CMW®, which contains barium sulphate (BaSO₄) caused more bone resorption than Palacos®, which contains zirconium dioxide (ZrO₂), in an in vitro assay. Radiolucent bone cement particles caused less bone resorption than either. However, the proprietary bone cements differ in their chemical constituents by more than just the radio-opacifier they contain.

In order to investigate the effects of radio-opaque agents in bone cement particles at the bone-implant interface, we have used an in vivo rat-pin model for aseptic loosening. To reduce confounding factors, the particles were produced from one ‘base’ bone cement to which radio-opaque agents were added, so that these were the only differences between the particle types.

MATERIALS AND METHODS

Particles were produced from Palacos® (Schering-Plough, Welwyn, UK) without radio-opacifier, Palacos® with 15.6% w/w ZrO₂ and Palacos® with 15.6% w/w BaSO₄ by grinding 2 cm blocks of the cement against a 20μm diamond grinding disc on a Rotopol-21 grinding/polishing machine (Struers, Rodovre, Denmark) with constant glycerol irrigation. Particles were characterized using light microscopy at 250x magnification, image analysis and scanning electron microscopy. They were described in terms of their equivalent circle diameter (ECD), i.e., the diameter of a circle having the same area as the measured feature. Contamination from the manufacturing process was excluded using energy dispersive x-ray analysis and from bacteria by 72 hour microbiological culture. The Limulus Ameobocyte Lysate (LAL) assay (Bio*Whittaker, Wokingham, UK) was performed to assess endotoxin contamination. Particles were sterilized with gamma irradiation.

Aluminium oxide pins were implanted into the proximal right tibias of forty-eight adult, male, Sprague-Dawley rats so that the head of the pin lay flush with the tibial plateau. The rats were randomly allocated into four groups. Group 1 received intra-articular injections of 100μl (10⁶ particles) of the Palacos® without radio-opacifier (‘plain’) particle suspension into the operated knee at 8, 10 and 12 weeks following implantation of the pin. Group 2 received Palacos® with BaSO₄ particles and group 3 Palacos® with ZrO₂. The fourth group was given injections of 100 μl of 2% w/v Sprague-Dawley serum as a control. All the animals were killed 2 weeks after their last injection.

The operated tibias were removed en bloc, with two samples of synovial tissue from each knee being sent for microbiological culture. The tibias were fixed in 4% paraformaldehyde, dehydrated in alcohol, defatted in xylene and infiltrated with methylmethacrylate resin. 250μm longitudinal sections were cut through the bone and the pin, and polished and stained with toluidine blue. Histomorphometry was performed using light microscopy and image analysis software without prior knowledge of the type of injection the animal had received. The total area of gaps between pin and bone, including any fibrous tissue, was measured around each pin.

The total area of gap around the pins in each of the four groups were tested for normality using the Shapiro-Wilk method. A one-way analysis of variance (ANOVA) was performed to ascertain whether there was a difference between the four groups and post-hoc testing, using Tukey’s method, to isolate where any differences lay.

RESULTS

Over 2000 particles of each type were measured. All three groups had mean ECDs between 1.22 and 1.32μm, almost half were submicrometer in size and none exceeded 6μm. No bacterial or material contamination was detected by culture and EDX. The LAL assay revealed the endotoxin levels to be less than 0.18 Endotoxin Units/ml.

Nine pins had sunk below the level of the tibial plateau and had been sealed off from the knee joint by a layer of new bone. Regardless of injection group, they were associated with complete bony apposition to the pin and the absence of fibrous tissue or gap at the bone-implant interface. The remaining 39 pins were well positioned. There was bacterial growth (diphtheroids or enterococci) from synovial culture of four knees. In all four cases growth occurred in only one of the two culture bottles and there was no histological evidence of infection or gap areas above average for their particle injection groups. The bacteria were therefore considered to be contaminants from the retrieval process.

The gap areas of all four injection groups were found to be normally distributed by Shapiro-Wilk testing. The BaSO₄ group had the largest mean gap area (table) and some pins from this group were completely surrounded by fibrous tissue.

<table>
<thead>
<tr>
<th>Injection</th>
<th>Control</th>
<th>Plain</th>
<th>ZrO₂</th>
<th>BaSO₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gap mm² ± sd</td>
<td>0.96 ± 0.61</td>
<td>1.72 ± 0.83</td>
<td>2.36 ± 0.88</td>
<td>3.76 ± 3.54</td>
</tr>
</tbody>
</table>

There was a statistically significant difference between the four groups by ANOVA (p=0.02) but only the BaSO₄ group had a significantly larger gap area than the control group on post-hoc testing with Tukey’s method (p=0.01). Whilst failing to reach statistical significance, there was a distinct trend of reducing similarity between the ‘plain’ and ZrO₂ groups (p=0.887), the ZrO₂ and BaSO₄ groups (p=0.357) and the BaSO₄ and plain groups (p=0.13).

DISCUSSION

This study sought to investigate the effects of bone cement particles on the bone-implant interface using an intra-articular in vivo model. The particles we have produced are very similar in size to those found in retrieval studies and we believe them to be clinically relevant.

Only bone cement particles containing BaSO₄ caused a significantly larger gap around the pin than vehicle control. Both plain particles and those containing ZrO₂ produced larger gaps than the control but this did not reach statistical significance. Our study would lend support to the theory that the BaSO₄ in bone cements may be responsible for the increased bone resorption seen in vitro and would also accord with the clinical performance of bone cements reported in the Swedish Hip Registry.

BIBLIOGRAPHY