DOSE-DEPENDENT REDUCTION OF BONE INDUCTIVE PROPERTIES BY ETHYLENE OXIDE

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Sterilisation of demineralised bone matrix with ethylene oxide has been claimed to destroy the ability of bone matrix to induce new bone formation on intramuscular implantation. Other workers have routinely used ethylene oxide sterilised bone matrix for assays in rodents without detrimental effects. We studied the effects of various lengths of exposure to ethylene oxide gas, and found that bone induction properties are destroyed in a dose-dependent manner. After a short exposure, bone induction properties were moderately diminished. However, this short ethylene oxide treatment did not kill Bacillus subtilis spores. A sterilisation procedure that killed these spores rendered the implants incapable of bone-induction.

Bone induction is a process whereby mesenchymal cells, that would otherwise not give rise to cartilage or bone, are induced by local factors to multiply and differentiate into cartilage-forming and bone-forming cells. This process can be elicited by factors present in demineralised bone matrix and has mainly been studied by the implantation of demineralised bone matrix in laboratory animals and man. Clinical success with demineralised bone matrix implantation has been reported (Glowacki et al 1981; Iwata et al 1981; Urist and Dawson 1981). In several reports, human bone matrix implants were sterilised with ethylene oxide (Iwata et al 1981; Kakiuchi et al 1985; Ousterhout 1985). In several rodent studies, before the clinical use of demineralised bone, ethylene oxide sterilised implants were used routinely without obvious detrimental effects (Syftestad and Urist 1982; Glowacki and Mulliken 1985). However, ethylene oxide has been reported to totally destroy bone induction properties (Munting et al 1988). Human ethylene oxide sterilised bone matrix is currently available from bone banks.

The confusion about the effect of ethylene oxide on bone induction potential mars the interpretation of most clinical results of demineralised bone matrix implantation. We investigated whether the differing findings following ethylene oxide sterilisation reflect the degree of exposure to ethylene oxide.

MATERIAL AND METHODS

Animals. Female Sprague-Dawley rats were obtained from Mellegård avelslaboratoriet (Copenhagen, Denmark) and kept for one week before the experiments started. The animals were divided into three groups. In each group, half the rats were killed to provide implants for the others. Body-weights at the start of the experiment corresponded to those at about 60 days of age.

Matrix preparation. Femur diaphyses were collected from donor rats and immediately cleaned from periosteum and marrow. They were kept as pairs from each donor in sterile glass tubes, defatted with 12 ml chloroform methanol 1:1 for two hours at room temperature, rinsed in methanol, demineralised in 12 ml 0.6 N HCl for 48 hours at room temperature with three changes, rinsed five times with sterile deionised water, lyophilised and finally weighed. The implants were about 8 mm long, 3 mm wide tubes, which became soft after rehydration. Samples were checked for completeness of demineralisation, and contained less than 1 µg calcium per mg dry matrix.

Sterilisation. One implant in each pair was treated with ethylene oxide and the other was kept as an untreated control. The ethylene oxide treatment was performed with a 'Steri-Vac' 4XL gas-steriliser (3M Company, St. Paul, Minnesota). The sterilisation temperature was 37°C and the humidification time was 30 minutes for all three procedures. The process was monitored each time by six samples of B. subtilis 10^9 ('Attest' 1264; 3M Company) incubated at 37°C within 10 minutes of cycle completion. Incubation was for 48 hours and bacterial growth was indicated by a yellow colour read-out. Three exposure times were used: the standard cycle with 240 minutes; 30 minutes; and five minutes.

Evaluation. The rats were killed after 21 days. The specimens were dissected,ashed in a muffle furnace (800°C, 24 hours) and dissolved in 1.5 ml of 6 mol/l HCl.
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Table I. Calcium yield per implanted dry matrix weight (μg/mg) from rats each receiving one ethylene oxide treated and one control implant (mean, SD)

<table>
<thead>
<tr>
<th>Ethylene oxide exposure (minutes)</th>
<th>5 (n=11)</th>
<th>30 (n=11)</th>
<th>240 (n=12)</th>
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<tbody>
<tr>
<td>Ethylene oxide</td>
<td></td>
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<tr>
<td></td>
<td>17.0 (17.6)</td>
<td>11.4 (5.3)</td>
<td>1.0 (0.4)</td>
</tr>
<tr>
<td>Control</td>
<td>28.1 (11.7)</td>
<td>39.0 (14.4)</td>
<td>29.1 (17.8)</td>
</tr>
<tr>
<td>Ratio</td>
<td>0.62 (0.19)</td>
<td>0.32 (0.16)</td>
<td>0.05 (0.03)</td>
</tr>
<tr>
<td>Number of positive B. subtilis cultures</td>
<td>6 of 6</td>
<td>6 of 6</td>
<td>0 of 6</td>
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</tbody>
</table>

The acid was evaporated in a vacuum centrifuge and the specimens redissolved. Calcium was measured in a ‘Dacos’ machine with the thymol blue reaction. With this method, non-ductive matrix implants may yield 2.2 (SD 0.8) μg calcium per mg implanted dry matrix after six weeks (Aspenberg and Andolf 1989).

RESULTS

Results are given in Table I. Ethylene oxide treatment for five minutes reduced the bone yield to 62% of that of the control implants (p < 0.003), but did not kill the B. subtilis spores. Treatment for 30 minutes reduced the bone yield to 32%, and the spores still survived. After 240 minutes treatment, no bone induction was demonstrated and the B. subtilis spores were killed.

DISCUSSION

The results indicate that a short sterilisation procedure with ethylene oxide may not destroy bone induction properties, although it may perhaps reduce bacterial numbers sufficiently for animal experiments. This is in accordance with reports in which an unspecified ethylene oxide treatment has preceded the implantation of bone matrix or extracted proteins in laboratory animals (Syftestad and Urist 1982; Glowacki and Mulliken 1985; Kawamura and Urist 1988).

On the other hand, when the sterilisation procedure is such that the demands for clinical use are met (that is, complete elimination of spores), the bone induction properties of the implant are lost, as shown by Munting et al (1988). Such thorough sterilisation procedures have probably been used in studies of ethylene oxide-sterilised demineralised bone matrix in man. Therefore, the reported positive results in man are probably due to effects other than bone induction, such as osteoconduction. This is suggested by various clinical observations such as slowly progressive ingrowth into the implanted material (Kakiuchi et al 1988).

The exposure times we used should not be expected to produce identical effects on other types of bone matrix specimens, since the effect is dependent on gas penetration which may vary with factors such as porosity, size and shape of the specimen.

There are only a few studies in man which have used other sterilisation procedures – namely, radiation. Since radiation is also suspected to destroy bone induction properties (Buring and Urist 1967; Urist and Hernandez 1974; Munting et al 1988), we cannot be certain about bone induction in man. Further, it is not clear that bone formation can be induced in man with the same type of implants that are effective in rodents. In other primates such as squirrel monkeys, allogenous or autogenous bone matrix does not induce extraskeletal bone formation, although the same implants induce bone formation in athymic rats (Aspenberg, Lohmander and Thorngren 1988, 1990).

Pure inductive proteins on appropriate carriers may become available. The drawbacks of sterilisation may then be eliminated and, hopefully, the induction potency will be higher than that of demineralised bone matrix.

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REFERENCES


