Influence of aeration, storage, and rinsing conditions on residual ethylene oxide in freeze-dried bone allograft

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Abstract Bone allografts sterilized with ethylene oxide gas (EO) are used in the field of orthopedic surgery, and the reduction of the EO residual concentration is an urgent clinical matter. We therefore investigated the efficacy of aeration and the effects of varied preservation periods and rinsing conditions on the reduction of EO residuals in freeze-dried bone allografts in the present study. Before aeration, the EO residual level was 12.6 ppm, and, after the repeating of aeration at 60°C once, two times, and three times, the level decreased to 10.9 ppm, 3.1 ppm, and 0.47 ppm, respectively. Regarding the duration of preservation at room temperature, the mean EO residual level was 10.5 ppm, 4.9 ppm, and 4.6 ppm, 1, 2, and 3 weeks after EO sterilization, respectively. By rinsing with physiological salt solution, the level was decreased to 6.9 ppm by 5-min rinsing with 100 ml. Rinsing with 500 ml of this solution decreased the levels to 3.9 ppm, 2.8 ppm, and 2.0 ppm when done for 1, 5, and 10 min, respectively. Rinsing with 2000 ml of this solution decreased the levels to 3.6 ppm, 2.6 ppm, and 1.7 ppm when done for 1, 5, and 10 min, respectively. These experimental results with chip bone allografts lead us to recommend repeated preoperative aeration and more than 2 weeks’ preservation before use for reducing the residual EO concentration. It was also evident that intraoperative rinsing with 500 ml of physiological saline for 10 min reduced the EO residual level.

Key words Freeze-dried bone · Ethylene oxide gas · Sterilization · Bone grafting

Introduction

Allografts have been used in orthopedic surgery for many years. Autoclaving is known to be unsuitable for the sterilization of bone allografts, because it may denature structural and bioactive proteins, including collagen and bone morphogenetic proteins. Another method of sterilization of medical supplies, with ethylene oxide (EO) gas, is widely used in hospitals and in the medical products industry; although freeze-dried bone is not used much for allografts in Japan, EO gas sterilization is now frequently used in the manufacturing process of this bone. Zhang et al. have reported that bone induction was little affected clinically by EO gas sterilization at 40°C. We have been using allografts that were defatted with chloroform and methanol, freeze-dried, and sterilized with EO gas in various procedures of orthopedic surgery since 1994.

Exposure to EO gas is accepted as an effective sterilization method for bone transplants, because EO completely penetrates into bone and destroys bacteria and viruses. The sterilization effect of EO gas is based on the acylation of amino acids. Because of the potential carcinogenicity of EO and its products, the reduction of the EO residual concentration in allografts is an urgent problem. EO gas is very soluble in both water and fat. Defatting and freeze-drying before EO exposure result in lower residual concentrations than freeze-drying after EO exposure. However, exposure to EO residuals has been reported to cause adverse changes in the human body.

We therefore investigated the efficacy of aeration and the effects of various preservation periods and rinsing conditions on the reduction of EO residuals in freeze-dried bone allografts in the present study.

Materials and methods

Cancellous femoral head specimens obtained upon surgical operations from patients with femoral neck fracture were stored at −80°C. All these bone donors were confirmed to be free of detectable bacterial and viral infections and other systemic diseases, except for ischemic disorders. The frozen cancellous bones were thawed in deionized water and cleaned of soft tissue and
cartilage, cut into 5-mm cubes by using a microtome, and washed with deionized water for 3 days at 4°C, with stirring done several times. The bones were then defatted in a mixture of chloroform and methanol (1:1 v/v) for 24 h at 4°C, and kept in flowing air for 24 h at room temperature to remove the residual chloroform and methanol by evaporation. The defatted bones were washed with sterile distilled water, with stirring, for 4°C, for 24 h at 4°C, frozen at −80°C, dehydrated by freeze-drying for 7 days, and then double-wrapped in sterilizing packs (ELK bag; ELK Corporation, Osaka, Japan) in which one side was paper and the other plastic film. The packs containing the cancellous bones were exposed to EO gas at a concentration of 6000 ppm for sterilization, for 4 h at 60°C, using an Eogelk SA300 sterilizer (ELK Corporation). These packs were then double-wrapped in an outer transparent waterproof pack made from polyethylene on both sides, and stored at room temperature until use.

The EO residual level in bone allografts is known to be affected by the frequency of preoperative aeration at 60°C, the duration of preoperative storage after sterilization, and the intraoperative rinsing of the allografts. In the present study, EO residual levels in freeze-dried cancellous bones were measured and comparatively studied after the bones had been treated under the experimental conditions described below. Ten samples were used for the experiment under each condition.

Experiment 1: effect of aeration on EO residual levels

Experiment 1 was designed to evaluate how the EO residual level is lowered by repeated aeration. This aeration means the ventilation of bone samples to forcibly remove EO residuals, using a gas-aerator in the sterilizing instrument, and taking 15 h for each aeration run. The EO residual concentrations in sterilized freeze-dried bones were measured before aeration, and after one, two, and three aeration runs at 60°C. EO residuals were assayed using a gas chromatograph (GC14; Shimadzu, Kyoto, Japan) as soon as possible after the following procedures. A freeze-dried bone sample, 5 g, and 50 ml of 50% ethanol were placed in a 50-ml flask, and the flask was tightly closed and immersed in a water bath (70 ± 2°C) for 3 h, with gentle shaking. Ten ml of the extraction fluid was collected and stored in a tightly sealed container at 3°C until the gas chromatographic assay. The results were expressed as ppm. Analysis of variance (ANOVA) was used for statistical comparisons.

Experiment 2: effect of preservation period on residual EO levels

EO residual levels in the freeze-dried sterile bone samples were measured after preservation at room temperature for various periods, namely, immediately after, and 1, 2, and 3 weeks after EO gas sterilization.

Experiment 3: effect of rinsing on EO residual levels

Utilizing the good solubility of EO in water, we rinsed the freeze-dried sterile bone samples repeatedly with physiological salt solution; namely, with 100 ml for 5 min, and with 500 ml and 2000 ml for 1, 5, and 10 min for each volume; the EO residual levels were measured after every rinsing.

Results

EO residual levels in the freeze-dried sterile bone samples immediately after EO gas sterilization, without aeration and rinsing (control group), ranged from 11.8 ppm to 13.6 ppm (mean, 12.6 ppm). By repeating aeration one, two, and three times, the EO residual levels decreased to 9.3–13.3 ppm (mean, 10.9 ppm), 1.6–4.7 ppm (mean, 3.1 ppm), and 0.0–1.9 ppm (mean, 0.47 ppm), respectively. Significant decreases were observed between the first and second aeration runs, and between the second and third aeration runs (Fig. 1). Each P value was less than 0.01.

Regarding the duration of preservation, the mean EO residual levels were 10.5 ppm (range, 2.3–15.5 ppm), 4.9 ppm (range, 1.0–12.8 ppm), and 4.6 ppm (range, 0.0–10.4 ppm) 1, 2, and 3 weeks after EO gas sterilization, respectively. A significant difference was observed between the values 1 and 2 weeks after the sterilization, and the P value was less than 0.05, but no significant

![Fig. 1. Effect of aeration on ethylene oxide (EO) residual levels. Significant decreases in EO residual levels were observed between the first and second aeration runs and between the second and third aeration runs. Levels of significance, using analysis of variance (ANOVA), are indicated. Bars show standard error](image-url)