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ORIGINAL ARTICLE

The effect of hydrogen gas evolution of magnesium implant on the postimplantation mortality of rats

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Summary *Background/Objective:* Hydrogen gas cavity is formed during *in vivo* degradation of magnesium implants. In many studies, the gas cavity is mostly punctured out subcutaneously. However, this procedure becomes inapplicable in certain internal surgeries; therefore, the effect of this gas cavity is worth further assessment.
Methods: In this study, we investigated the effect of hydrogen gas evolution on the mortality of rats and analysed the whole body capacity to relieve the gas. Porous pure-magnesium implants were implanted in the femoral bone defect of adult Sprague-Dawley rats up to 18 days, and their survival rate was calculated while the gas cavity size was measured, and its effect was analysed with support of radiographic and blood analysis.
Results: The gas cavity was rapidly formed surrounding the implantation site and obviously decreased the rats' survival rate. The gas was observed to swell the surrounding implantation site by filling the loose compartments and then dispersing subcutaneously to other areas.
Conclusion: The rat's whole body capacity was unable to tolerate the rapid and persistent hydrogen gas cavity formation as shown by high postimplantation mortality.

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Introduction

Magnesium and its alloys have been extensively studied as potential biodegradable metals for bone temporary implants [1–5]. Despite their proven suitable compatibility to bone tissue [6–8], the nature of magnesium degradation is associated with hydrogen gas evolution, which forms gas cavities in the surrounding implantation tissue [9,10]. Overall, magnesium degrades *in vivo* via the corrosion reaction: $Mg + 2H_2O \rightarrow Mg(OH)_2 + H_2$, which shows that 1 g of pure magnesium produces about 1 L of hydrogen gas. Once the local hydrogen saturation of blood and tissues are exceeded, diffusion and solubility of hydrogen in local biological tissues are hindered. Hydrogen gas then accumulates in tissue cavities [6,11]. A study has shown that hydrogen was not the major composition of the gas cavity in mice [10]. Most animal implantation studies on magnesium implants dealt with this subcutaneous gas cavity by puncturing the gas out [6,12]. However, this procedure may not be applicable for certain internal surgeries such as intraosseous pins and endovascular stents.

A small gas cavity may have little effect on the body system, as this gas is quickly exchanged with the surrounding tissue [10], but the effect of excessive gas cavity is yet to be ascertained as it may be harmful. Excessive hydrogen gas evolution creates pressure that induces some mechanical disturbances of bone regeneration resulting in distinct callus formation [9]. After all, this gas cavity formation was the main reason for which magnesium was abandoned in early usage [3]. Therefore, this study aims to analyse the whole body capacity of rats to relieve hydrogen gas from a magnesium implant and to investigate the effect of hydrogen gas evolution on its mortality. Implants were made from sintered magnesium powders; thereby, their porous structure allows for a fast degradation and excessive hydrogen evolution. Gas cavity formation was observed under radiography and survival rate of the rats was calculated.

Materials and methods

Porous magnesium implant preparation

Closed-porous pure magnesium implants (diameter 13 mm, thickness 2.5 mm, weight 0.24–0.26 g, porosity <5%) were prepared via powder sintering process. Commercial purity pure magnesium powders (< 100 μm) were uniaxially pressed under 13.8 MPa into tablets, and then sintered under argon at 400 °C for 1 hour and cooled to room temperature. The sintered tablets (implants) were sterilised with a hot dry air oven at 160 °C and UV light for 60 minutes prior to implantation. Detailed characterisation and testing of the material were not performed as they are not the focus of this study.

Animal preparation and implantation procedure

Twenty adult Sprague-Dawley rats (weight 147 ± 10 g, age 12 weeks) were used in this study with ethical clearance from the Animal Care and Use Ethics Committee of Bogor Agricultural University (ACUC No: 6-2014 IPB). The implant weight accounted for ~1.7% of the rat's weight, which can be considered high. All rats were acclimatized for 2 weeks before the study with oral administration of acclimatization drugs with 10 mg/kg antibiotic (Claneksi, PT. Sanbe Farma, Jakarta, Indonesia) for 5 days, 10 mg/kg anthelmintic (Univerm, VMD, Budapest, Hungary) twice before and after antibiotic administration, and 20 mg/kg antiprozoa (Flagyl, Oubari Pharma, Damascus, Syria) for 5 days. All rats were anesthetised intraperitoneously by using 50 mg/kg of ketamin hydrochloric acid combined with 5 mg/kg of xylazine hydrochloric acid (Ilium, Troy Laboratories, Glendenning, Australia). Right lateral femoral hair were then clipped and desinfected by using 70% alcohol and 10% povidone iodine prior to surgery. The skin was incised and the femoral muscle was retracted until the femur bone reached. The implant was inserted into flatten bone defects drilled at the femur bone on latero-medial region. The sham group was treated with the same surgical procedure but without implantation of magnesium and thus served as the control group. Femoral muscle and skin were then sutured by using 5/0 synthetic absorbable polyglactin suture (Hinglact, HiCare, Kerala, India).

Postimplantation observation and analysis

Death of the rats was noted and a survival rate was calculated in term of percent mortality up to Day 18 postimplantation. Body swelling as an indication of gas cavity formation was directly measured by using a calliper for its length in longitudinal and transversal directions (in cm) throughout the implantation period. The gas cavity was also observed by using a portable digital computer radiography (CR7 Vet Digital X-Ray, iM3 Inc., USA) at Day 7 and Day 14 postimplantation. Radiodensity was further analysed with the help of an image analysis software (ImageJ, NIH, USA). Peripheral blood profile was monitored before implantation and at Day 7 postimplantation by collecting 0.5 mL blood sample from the tail venous of each rat and placing in ethylenediaminetetraacetic acid vacutainer for blood parameters analysis. Statistical analysis was done by using a one-way analysis of variance with a post hoc Duncan test using SPSS v.16.0 software (SPSS Inc., USA) at a 95% confidence level. A value of $p < 0.05$ was considered as statistically significant.

Results

Figure 1 shows the magnesium tablet implantation process, survival rate curves, and gas cavity formation. Gas cavity was rapidly formed around the implantation site and obviously visible. A smooth skin bulging was observed and the skin palpation produced sensation of gas cavity during observation time at Day 7 postimplantation (Figures 1C and 1D). The number of survived rats was rapidly decreasing with no survival at Day 18 postimplantation.

Figure 2 shows the size of gas cavities and its distribution in different part of the rat's body measured throughout the implantation period. The gas cavity size reached its maximum at Day 5 and was decreasing in the following implantation days (Figure 2A). The gas cavity was spreading