RESEARCH ARTICLE

Serum IL-6 AS A marker of immuno-inflammatory response to Zn-Mg alloy implants in porcine (*Sus scrofa*)

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Abstract

Background and Aim: The present study aimed to evaluate the immune-inflammatory response induced by ZnMg(3x) and ZnMg(4x) alloy implants in the porcine urinary bladder by measuring serum interleukin-6 (IL-6) levels.

Materials and Methods: Nine porcines, aged 2-3 months old with 25-30 kg body weight, were divided into three groups: ZnMg(3x), ZnMg(4x), and control, while the observation time was performed on days 0, 14, and 28. Implantation was performed on the urinary bladder using cystotomy techniques. Measurement of IL-6 levels is done using enzyme-linked immunosorbent assay (ELISA). Data were analyzed statistically using analysis of variance (ANOVA).

Results: The results showed that at day 0 for the ZnMg(3x) and ZnMg(4x) implants were 0.53 ± 0.92 pg/mL and 0.10 ± 0.17 pg/mL, respectively. On day 14, IL-6 levels in both implants decreased, although not significantly. On day 28, a slight increase was observed in ZnMg(3x) compared to ZnMg(4x), but this was also not significant ($P>0.05$).

Conclusion: Based on the results, Zn-Mg materials are suitable to be used as urinary stent base materials.

Keywords: IL-6, implants, Zn-Mg, porcine

Introduction

Biomaterials used as implants in the medical field have developed rapidly over the past few decades. Currently, implants are not only used in orthopedics but also in urology, where they serve not only as catheters and slings in the urinary tract, but also play a role in reconstructing organ morphology and tissue. In addition, they are utilized in various reconstructive techniques to address abnormal conditions (Elsaway & Achala, 2017). Ideally, a ureteral stent should have properties that maintain urine flow to ensure optimal upper urinary tract drainage, resist encrustation, prevent infections, and be biodegradable (Lange *et al*., 2015).

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The Science and Technology Policy Institute (2000) explains that currently used biomaterials have classified into five categories: polymers (commonly used for catheters), composites, metals and alloys, ceramics (often combined with metals), and biological materials. There are three groups of metals developed as biodegradable materials: magnesium (Mg), iron (Fe) alloys, and zinc (Zn) alloys. These metals are expected to exhibit gradual in vivo corrosion and fully dissolve once tissue healing is complete (Li *et al*., 2014). Biodegradable materials can reduce the need for repeated surgical procedures to remove implants after healing.

The implants used must have good biocompatibility, which refers to the ability of the host to respond to a biomaterial without causing adverse effects (Williams, 2008). Implantation can initiate acute inflammatory responses primarily by activating phagocytic cells, promoting chemotaxis, and facilitating the adhesion of proteins to the implant surface. After immune cells have recruited, any debris particles released from biomaterials are engulfed by macrophages through phagocytosis. This process subsequently triggers the release of a series of pro-inflammatory mediators, including IL-1β, tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and IL-8 (Christensen *et al*., 2017; Samelko *et al*., 2021). Interleukin-6 is a pro-inflammatory cytokine with various biological activities, including acting as a substitute mediator for immunoglobulin, regulating the acute phase response, and as an indicator of inflammation in the body, both locally and systemically (Naseem *et al*., 2016).

Emme *et al*. (2015) reported that although Mg exhibits good biocompatibility, it is highly reactive in biological environments, resulting in a very high corrosion rate. One approach to reducing the corrosion rate of Mg without compromising its biocompatibility is by alloying it with other elements (Chen *et al*., 2014). Zinc is considered the primary alloying element for Mg due to its superior mechanical properties compared to Mg, which can help slow down the corrosion rate and enhance the overall durability of Mg-based materials (Levy *et al*., 2017). Based on the background, the present study aimed to evaluate the immune-inflammatory response induced by ZnMg(3x) and ZnMg(4x) alloy implants in the porcine urinary bladder by measuring serum IL-6 levels.

Materials and Methods

Research Procedure

Animal preparation

All procedures in this study were approved by the Animal Ethics Committee of IPB (Approval No: 152/KEH/SKE/X/2019). The study involved nine male and female porcines, aged 2–3 months, with body weights ranging from 25 to 30 kg. An acclimatization period of 14 days was conducted before treatment to ensure that all animals had the same health status**.** This study is divided into two implant groups, ZnMg(3x) and ZnMg(4x), as well as a control group. Each treatment group consists of 3 porcines.

Sample collection

Implantation of the device into the urinary bladder wall was performed using the cystotomy method. Blood samples were collected on days 0, 14, and 28. On day 0, blood was drawn 60 minutes after implantation. The collected blood was then centrifuged to obtain serum. The serum was subsequently tested for IL-6 concentration using the direct enzyme-linked immunosorbent assay (ELISA) method. Sample preparation, reagent handling, assay procedures, and result calculations followed the guidelines established by the manufacturer, Thermo Scientific™, the producer of the equipment used.

IL-6 assay procedure

All reagents, samples, and standards were prepared according to the instructions. Then, 100 µL of standard solution and samples were added into the wells, covered, and incubated at room temperature for 2 hours and 30 minutes. The plate cover was removed, and the plate was washed four times with wash buffer. Next, 100 µL of biotinylated antibody was added to each well, then the plate was covered and incubated at room temperature for 60 minutes. The plate was washed again with wash buffer four times. Afterward, 100 µL of streptavidin-HRP reagent was added to each well, the plate was covered, and incubated at room temperature for 45 minutes.

The plate was washed again with wash buffer four times. Subsequently, 100 µL of TMB substrate was added to all wells, and the plate was covered and incubated in the dark at room temperature for 30 minutes. Then, 50 µL of stop solution was added to each well. The final step was measuring the absorbance and calculating the results using an ELISA plate reader with a wavelength of 450 nm, generating a graph and equation (coefficient) obtained from the standard curve, which was then used to calculate the concentration values of each sample.

Research Methodology

This study is experimental research using analysis of variance (ANOVA). If significant differences are found, a Duncan test will be conducted at a 95% confidence interval. The data are presented as means with standard deviation and are described narratively.

Results and Discussion

Serum examination in porcine (*Sus scrofa*) showed that the mean IL-6 levels did not differ significantly among the ZnMg(3x), ZnMg(4x), and control groups on days 0, 14, and 28 ($P > 0.05$). The mean IL-6 levels are presented in Table 1.

Table 1. Mean (±SD) Serum IL-6 levels (pg/mL) in Porcine after Implantation with ZnMg(3x) and ZnMg(4x) alloys

Observation time (Day to-)	Implant groups		
	ZnMg(3x)	ZnMg(4x)	Control
0	0.53 ± 0.92^{a}	0.10 ± 0.17^{a}	0
14	0.44 ± 0.77^{a}	0^{a}	0
28	0.68 ± 0.60^{ab}	0^{a}	0

The superscript letters in different rows and columns indicate no significant differences ($P>0.05$)

Based on Table 1, the average IL-6 levels on day 0 for the ZnMg(3x) and ZnMg(4x) implants were 0.53 ± 0.92 pg/mL and 0.10 ± 0.17 pg/mL, respectively. On day 14, IL-6 levels in both implants decreased, although not statistically significant. On day 28, a slight increase was observed in ZnMg(3x) compared to ZnMg(4x), but this was also not significant. The comparison of these results suggests that the ZnMg(4x) group had a better reduction in IL-6 levels compared to the ZnMg(3x) group.

The IL-6 levels on day 0 in this study represented the body's response to the implant. It is similar to Anderson *et al*. (2008), who stated that the body's response following implantation triggers a series of continuous processes, including tissue injury, blood-implant interaction, acute inflammation, chronic inflammation, foreign body reaction, and fibrosis. Metal implants involve immune reactions, both innate and adaptive, which can activate acute inflammatory responses primarily through phagocyte activation, chemotaxis, and protein adhesion to the implant surface (Chen *et al*., 2018). One of the pro-inflammatory mediators released during the phagocytosis process is IL-6. Interleukin-6 induces the differentiation of T helper 17 (Th17) cells, which play a key role in interaction with metal implants as part of the adaptive immune response (Khodaei *et al*., 2023).

IL-6 is a pro-inflammatory cytokine with multiple functions and is produced by various cell types, such as immune cells, fibroblasts, endothelial cells, adipocytes, and myocytes (Kayaalti *et al*., 2011). Based on the results, IL-6 levels on day 0 were higher on days 14 and 28. It is because the peak concentration of IL-6 occurs within 2–48 hours post-surgery and begins to decline within 48–72 hours (Shaw & Martin, 2009). According to Gabay (2006), the function of IL-6 release during the acute phase response is to suppress the production of pro-inflammatory cytokines without inducing the release of anti-inflammatory cytokines. In addition, IL-6 stimulates the production of IL-1 receptor antagonists, which act as anti-inflammatory mediators. In other words, IL-6 plays a protective role during the acute phase response.

The study showed a decrease in IL-6 levels at all observation times, with the ZnMg(4x) group demonstrating a more significant reduction in IL-6 levels compared to the ZnMg(3x) group. It may be due to the concentration of Zn in the tissue, which is a crucial factor that must remain within an optimal range. According to Levy *et al*. (2017), high Zn concentrations can change intracellular signaling pathways, which may promote oxidative stress and lead to apoptosis or necrosis of cells in the surrounding tissue. In addition, Jin *et al*. (2017) stated that compared to pure Zn, the low-alloy Zn-xMg implants can lead to more inflammatory cells.

One of the primary concerns associated with using Zn-based implants is their long-term degradation profile, which may lead to the sustained presence of corrosion by-products and consequently increase the risk of foreign body reactions (Shao *et al*., 2022). In addition, as reported in several previous studies, implants are recognized as foreign materials by the immune system, which can trigger inflammatory responses upon implantation. Therefore, the development and utilization of biostable metal alloys are necessary to minimize these inflammatory reactions.

In this study, Zn-Mg alloy was implanted into the urinary bladder wall of porcine and observed for a month post-implantation. The findings demonstrated no toxicity or chronic inflammation, as evidenced by IL-6 levels decreased on days 14 and 28. This statement is consistent with previous studies indicating that levels of TNF-α and IL-6 tend to increase in patients experiencing implant failure (Bottner *et al*., 2007).

Zinc alloy materials possess superior mechanical strength, stable corrosion rates, and high biocompatibility. In addition, Zn is an essential element in physiological systems and plays a role in both the innate and adaptive immune system (Venezuella *et al*., 2019). Another advantage of Zn-based implants is that the corrosion process of this material does not produce gas, unlike Mg-based implants. As a result, Zn-Mg alloys exhibit a lower corrosion rate and generate less gas compared to other binary alloys.

(Gu and Zheng, 2010; Levy *et al*., 2017). Based on the results, Zn-Mg materials are not immunostimulatory and suitable for urinary stent base materials.

Conclusion

The present study demonstrated that the implantation of Zn-Mg alloys, particularly ZnMg(4x), did not elicit a significant increase in serum IL-6 levels in porcine over 28 days, indicating a favorable biocompatibility profile. The observed decline in IL-6 levels on days 14 and 28 suggests a transient inflammatory response consistent with the acute phase of post-implantation healing. The ZnMg(4x) group exhibited a more pronounced reduction in IL-6, indicating potentially superior anti-inflammatory properties compared to ZnMg(3x). These findings support the biocompatibility of Zn-Mg alloys, particularly ZnMg(4x), and their potential application as biodegradable implant materials, such as urinary stent bases, due to their minimal pro-inflammatory effects and lack of chronic inflammatory response.

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