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THE EFFECT OF HA-COATED Mg ZK60 SCREWS ON CARDIOVASCULAR FUNCTION IN EXPERIMENTAL ANIMALS

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ABSTRACT

Objective: *To evaluate the effects of hydroxyapatite (HA)-coated Mg ZK60 screws on cardiovascular function when implanted into the femurs of experimental animals.*

Materials and methods: *A prospective, experimental cross-sectional study was conducted on 84 healthy white rabbits, randomly divided into 3 groups (Group 1: 28 rabbits implanted with HA-coated Mg ZK60 screws; Group 2: 28 rabbits implanted with uncoated Mg ZK60 screws; Group 3: 28 rabbits implanted with titanium screws). Cardiovascular function was assessed via heart rate, P-wave duration and amplitude, R-wave amplitude, QRS complex duration, and T-wave amplitude before surgery and at 3, 7, 30, 60, 90, and 180 days postoperatively. The study was carried out at the Department of Pathophysiology, Vietnam Military Medical Academy.*

Results: *There were no statistically significant differences in heart rate, P-wave duration and amplitude, R-wave amplitude, QRS duration, and T-wave amplitude before and after implant surgery, as well as between the groups at each follow-up time point ($p > 0.05$).*

Conclusion: *HA-coated Mg ZK60 screws and the control screw groups do not affect the cardiovascular function of experimental rabbits when implanted into their femurs.*

Keywords: ECG, rabbits, HA-coated Mg ZK60 screws

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1. INTRODUCTION

To date, most bone fixation devices used in treatment require a second surgical procedure for removal once the bone has completely healed. Consequently, patients typically must undergo two operations (initial bone fixation and subsequent device removal). This not only impacts patient health but also increases treatment costs and places a financial burden on the healthcare system. Therefore, researching and developing biodegradable bone fixation materials is essential. Among potential biodegradable options, magnesium (Mg)-based structural materials have attracted significant attention due to their favorable properties for bone fixation applications, particularly the Mg ZK60 alloy [1]. However, its excessively rapid degradation rate remains a major limitation, preventing its widespread clinical use. To address this drawback, researchers have proposed various solutions, including coating the material surface with hydroxyapatite (HA) to enhance biocompatibility and slow down the degradation rate.

To establish a foundation for further research toward the clinical application of HA-coated Mg ZK60 materials, evaluating biocompatibility is a crucial initial step. Specifically, assessing the effects of the material on organ functions-especially the cardiovascular system-is of vital importance in experimental studies.

Therefore, we conducted this study to evaluate the biocompatibility of HA-coated Mg ZK60 bone fixation materials on cardiovascular function in experimental animals.

2. SUBJECTS AND METHODS

2.1. Subjects

84 healthy, adult, purebred white rabbits weighing 1.8-2.3 kg each were used in this study. The rabbits were supplied by the Animal Center of Vietnam Military Medical University and met all

criteria for experimental animals. The study was conducted at the Department of Pathophysiology, Vietnam Military Medical University, from August 2021 to October 2023.

2.2. Methods

Study design: A prospective, experimental cross-sectional study.Study sample size: Calculated based on the guidelines by Charan and Kantharia (2013) [2]. Accordingly, the experimental sample size was estimated using the E index, where the formula is: E = Total number of animals used - Total number of groups; with E falling within the range of 10–20. Thus, with 3 groups, the required number of animals ranges from 13 to 23 for each study time point. This means that a minimum of 4 and a maximum of 8 animals per group are required at each study time point. Consequently, we selected a sample size of at least 7 rabbits per group for each study time point.

- Procedure: 84 rabbits were randomly divided into 3 groups, with 28 rabbits in each group:

+ Group 1: Consisted of 28 rabbits implanted with HA-coated Mg ZK60 screws.

+ Group 2: Consisted of 28 rabbits implanted with uncoated Mg ZK60 screws.

+ Group 3: Consisted of 28 rabbits implanted with titanium screws.

At 30, 60, 90, and 180 days post-implantation, 4 rabbits were randomly selected from each group, anesthetized, and euthanized to harvest the screws and surrounding soft tissues at the implantation site for histopathological evaluation. Consequently, the number of rabbits gradually decreased across the aforementioned follow-up time points.

- Surgical technique for screw fixation on experimental rabbit femurs:

+ The rabbit's femoral region was shaved and disinfected using 10% betadine solution and 70° alcohol. Anesthesia was induced and maintained via intravenous infusion of 1% propofol at a dose of 15–25 mg/kg/hour.

+ A 4-cm longitudinal skin incision was made in the anterolateral region of the rabbit's thigh. The subcutaneous tissue was dissected to expose the muscular interspace between the anterior and posterior thigh muscles. Dissection was continued along this interspace to locate and expose the femur. A periosteal elevator was used to completely strip the periosteum, and one cortex of the rabbit femur was transected.

+ A 4-hole plate was placed on the lateral surface of the rabbit femur, and the drilling positions corresponding to the plate holes were marked. A 1.8-mm diameter drill bit was used to drill through the femoral cortex at the marked positions. The plate was placed flush against the femoral cortex so that the plate holes aligned with the drilled holes, and the screws were tightened securely. The surgical site was closed in layers using medical sutures. Once the rabbits recovered from anesthesia, they were returned to their cages for further housing, care, and monitoring.

Assessment of rabbit cardiovascular function using electrocardiography (ECG): The rabbits were secured onto an animal restraint table, and their limbs were exposed. Needle electrodes (subcutaneous pin electrodes) were clipped to the limbs: the negative electrode was placed on the right forelimb, the positive electrode on the left hindlimb, and the neutral (ground) electrode on the right hindlimb. Continuous lead II ECG signals were recorded using the PowerLab system (ADInstruments, Australia). ECG data were analyzed offline using LabChart 8.0 software.ECG recordings were performed preoperatively and postoperatively at 3, 7, 30, 60, 90, and 180 days.

- Study parameters: Heart rate (beats per minute); P-wave duration and amplitude (ms and mV); R-wave amplitude (mV); QRS complex duration (ms); and T-wave amplitude (mV)

Data analysis: Statistical analysis was performed using SPSS 20.0 software, applying a two-way analysis of variance (ANOVA) with replication. Statistical significance was defined as $p < 0.05$.

3. RESULTS

Table 1. Heart rate before and after implant surgery

Follow-up time point	Group 1 (bpm)		Group 2 (bpm)		Group 3 (bpm)		p
	n	$\bar{X} \pm SD$	n	$\bar{X} \pm SD$	n	$\bar{X} \pm SD$	
Pre-surgery	28	255.24 ± 35.18	28	272.31 ± 35.32	28	273.76 ± 30.54	> 0.05
3 days post-surgery	28	261.15 ± 42.29	28	261.48 ± 23.65	28	264.35 ± 19.33	> 0.05
7 days post-surgery	28	257.79 ± 37.29	28	250.19 ± 30.12	28	264.79 ± 17.44	> 0.05

30 days post-surgery	28	271.05 ± 37.80	28	254.45 ± 29.15	28	262.29 ± 21.59	> 0.05
60 days post-surgery	21	258.51 ± 36.32	21	263.48 ± 20.19	21	266.68 ± 35.04	> 0.05
90 days post-surgery	14	274.77 ± 36.20	14	259.35 ± 38.09	14	274.77 ± 36.20	> 0.05
180 days post-surgery	7	283.41 ± 24.89	7	265.74 ± 4.30	7	263.44 ± 5.13	> 0.05
p	> 0.05		> 0.05		> 0.05		

There were no statistically significant differences in rabbit heart rate variations between the pre- and post-implantation periods, as well as among the groups at each follow-up time point (p > 0.05).

Table 2. P-wave amplitude before and after implant surgery

Follow-up time point	Group 1 (mV)		Group 2 (mV)		Group 3 (mV)		p
	n	$\bar{X}$ ± SD	n	$\bar{X}$ ± SD	n	$\bar{X}$ ± SD	
Pre-surgery	28	0.040 ± 0,060	28	0.021 ± 0,065	28	0.032 ± 0,056	> 0.05
3 days post-surgery	28	0.050 ± 0.063	28	0.046 ± 0.068	28	0.034 ± 0.031	> 0.05
7 days post-surgery	28	0.042 ± 0.033	28	0.027 ± 0.042	28	0.035 ± 0.034	> 0.05
30 days post-surgery	28	0.040 ± 0.035	28	0.047 ± 0.023	28	0.032 ± 0.015	> 0.05
60 days post-surgery	21	0.052 ± 0.030	21	0.050 ± 0.026	21	0.049 ± 0.016	> 0.05
90 days post-surgery	14	0.017 ± 0.039	14	0.021 ± 0.011	14	0.029 ± 0.021	> 0.05
180 days post-surgery	7	0.052 ± 0.037	7	0.052 ± 0.024	7	0.076 ± 0.002	> 0.05
p	> 0.05		> 0.05		> 0.05		

There were no statistically significant differences in P-wave amplitude variations between the pre- and post-implantation periods, as well as among the groups at each follow-up time point (p > 0.05).

Table 3. P-wave duration before and after implant surgery

Follow-up time point	Group 1 (seconds)		Group 2 (seconds)		Group 3 (seconds)		p
	n	$\bar{X}$ ± SD	n	$\bar{X}$ ± SD	n	$\bar{X}$ ± SD	
Pre-surgery	28	0.034 ± 0.005	28	0.033 ± 0.007	28	0.031 ± 0.004	> 0.05
3 days post-surgery	28	0.032 ± 0.006	28	0.032 ± 0.008	28	0.031 ± 0.006	> 0.05
7 days post-surgery	28	0.034 ± 0.005	28	0.032 ± 0.004	28	0.032 ± 0.005	> 0.05
30 days post-surgery	28	0.035 ± 0.004	28	0.034 ± 0.005	28	0.032 ± 0.005	> 0.05
60 days post-surgery	21	0.033 ± 0.005	21	0.031 ± 0.007	21	0.033 ± 0.006	> 0.05
90 days post-surgery	14	0.033 ± 0.003	14	0.031 ± 0.006	14	0.033 ± 0.004	> 0.05
180 days post-surgery	7	0.033 ± 0.001	7	0.033 ± 0.002	7	0.033 ± 0.002	> 0.05
p	> 0.05		> 0.05		> 0.05		

There were no statistically significant differences in P-wave duration variations between the pre- and post-implantation periods, as well as among the groups at each follow-up time point (p > 0.05).

Table 4. R-wave amplitude before and after implant surgery

Follow-up time point	Group 1 (mV)		Group 2 (mV)		Group 3 (mV)		p*
	n	$\bar{X}$ ± SD	n	$\bar{X}$ ± SD	n	$\bar{X}$ ± SD	
Pre-surgery	28	0.119 ± 0.170	28	0.102 ± 0.051	28	0.101 ± 0.071	> 0.05
3 days post-surgery	28	0.132 ± 0.135	28	0.106 ± 0.073	28	0.108 ± 0.076	> 0.05
7 days post-surgery	28	0.172 ± 0.107	28	0.123 ± 0.081	28	0.151 ± 0.111	> 0.05
30 days post-surgery	28	0.145 ± 0.060	28	0.155 ± 0.069	28	0.112 ± 0.062	> 0.05

60 days post-surgery	21	0.134 ± 0.068	21	0.090 ± 0.051	21	0.152 ± 0.026	> 0.05
90 days post-surgery	14	0.110 ± 0.059	14	0.122 ± 0.077	14	0.166 ± 0.079	> 0.05
180 days post-surgery	7	0.079 ± 0.052	7	0.131 ± 0.035	7	0.137 ± 0.014	> 0.05
p**	> 0.05		> 0.05		> 0.05		

There were no statistically significant differences in R-wave amplitude variations between the pre- and post-implantation periods, as well as among the groups at each follow-up time point (p > 0.05).

Table 5. QRS complex duration before and after surgery

Follow-up time point	Group 1 (second)		Group 2 (second)		Group 3 (second)		p
	n	$\bar{X}$ ± SD	n	$\bar{X}$ ± SD	n	$\bar{X}$ ± SD	
Pre-surgery	28	0.055 ± 0.014	28	0.055 ± 0.009	28	0.058 ± 0.017	> 0.05
3 days post-surgery	28	0.054 ± 0.011	28	0.058 ± 0.019	28	0.051 ± 0.010	> 0.05
7 days post-surgery	28	0.057 ± 0.013	28	0.058 ± 0.023	28	0.056 ± 0.009	> 0.05
30 days post-surgery	28	0.058 ± 0.013	28	0.055 ± 0.006	28	0.052 ± 0.006	> 0.05
60 days post-surgery	21	0.054 ± 0.007	21	0.052 ± 0.008	21	0.051 ± 0.006	> 0.05
90 days post-surgery	14	0.053 ± 0.010	14	0.051 ± 0.007	14	0.059 ± 0.009	> 0.05
180 days post-surgery	7	0.053 ± 0.012	7	0.054 ± 0.005	7	0.053 ± 0.002	> 0.05
p	> 0.05		> 0.05		> 0.05		

There were no statistically significant differences in QRS complex duration variations between the pre- and post-implantation periods, as well as among the groups at each follow-up time point (p > 0.05).

Table 6. T-wave amplitude before and after implant surgery

Follow-up time point	Group 1 (mV)		Group 2 (mV)		Group 3 (mV)		p
	n	$\bar{X}$ ± SD	n	$\bar{X}$ ± SD	n	$\bar{X}$ ± SD	
Pre-surgery	28	0.089 ± 0.137	28	0.070 ± 0.086	28	0.073 ± 0.089	> 0.05
3 days post-surgery	28	0.080 ± 0.061	28	0.071 ± 0.068	28	0.088 ± 0.053	> 0.05
7 days post-surgery	28	0.110 ± 0.126	28	0.067 ± 0.073	28	0.083 ± 0.048	> 0.05
30 days post-surgery	28	0.088 ± 0.059	28	0.077 ± 0.036	28	0.065 ± 0.060	> 0.05
60 days post-surgery	21	0.080 ± 0.051	21	0.075 ± 0.059	21	0.066 ± 0.044	> 0.05
90 days post-surgery	14	0.081 ± 0.068	14	0.075 ± 0.051	14	0.108 ± 0.091	> 0.05
180 days post-surgery	7	0.073 ± 0.030	7	0.067 ± 0.033	7	0.081 ± 0.042	> 0.05
p	> 0.05		> 0.05		> 0.05		

There were no statistically significant differences in T-wave amplitude variations between the pre- and post-implantation periods, as well as among the groups at each follow-up time point (p > 0.05).

4. DISCUSSION

Cardiovascular function in experimental animals is commonly evaluated through the values of waves and intervals on an electrocardiogram (ECG). In this study, we performed ECG recordings and analyzed ECG parameters using a continuous lead II configuration. Specifically, the analysis focused on several ECG indices related to atrial depolarization, ventricular depolarization, and ventricular repolarization. These are critical parameters for assessing cardiac function in the experimental animals.

On an animal electrocardiogram (ECG), the waveforms include the P-wave, QRS complex, T-wave, along with the PQ interval, ST segment, and QT interval. These waves and intervals have distinct clinical significances, reflecting the functions of the ventricles as well as the right and left atria. Specifically, the amplitude and duration of the P-wave represent the depolarization process of the atria (including both the right and left atria) [3]. Therefore, variations in P-wave amplitude and duration are correlated with changes in atrial function. The QRS complex, particularly the R-wave amplitude and QRS complex duration,

represents ventricular depolarization, while the T-wave represents ventricular repolarization [4]. Consequently, alterations in the parameters of the QRS complex and T-wave are associated with modifications in ventricular function.

The study results indicated that the implantation of HA-coated ZK60 screws, uncoated ZK60 screws, and titanium screws into the rabbit femur did not affect the atrial and ventricular depolarization or repolarization processes in the experimental rabbits (as evidenced by the electrocardiogram parameters). In this study, we also analyzed changes in rabbit heart rate before and after surgery. Heart rate is a crucial indicator for evaluating the intrinsic automaticity of the heart. The results showed no statistically significant differences between the preoperative heart rate and the postoperative heart rate following material implantation across all three experimental groups. Notably, the heart rate values and the wave durations on the ECG of all studied rabbit groups remained within normal physiological limits [4, 5].

Based on this knowledge, we assessed atrial and ventricular function by analyzing ECG parameters before and after implantation of HA-coated ZK60 screws, uncoated ZK60 screws, and titanium screws into the rabbit femur. The results showed no statistically significant differences in P-wave duration and amplitude before and after implantation. Similarly, QRS complex duration, R-wave amplitude, and T-wave amplitude did not differ significantly between the preoperative and postoperative time points.

The electrocardiogram (ECG) results demonstrated that both HA-coated and uncoated ZK60 screw systems had no adverse effects on the cardiovascular function of the experimental rabbits. All ECG variations recorded following the implantation of these HA-coated and uncoated ZK60 screw systems remained within safe physiological limits. Furthermore, both the HA-coated and uncoated ZK60 screw systems exhibited a cardiovascular safety profile in experimental rabbits equivalent to that of titanium screws-a material already proven safe in clinical practice

5. CONCLUSION

The study on the compatibility of the HA-coated Mg ZK60 bone fixation material with cardiovascular function in experimental rabbits demonstrated that ECG parameters-including heart rate, P-wave amplitude and duration, R-wave amplitude, QRS complex duration, and T-wave amplitude-showed no statistically significant differences between the rabbits implanted with HA-coated Mg ZK60 screws and those implanted with the control materials (uncoated Mg ZK60 and titanium). These parameters in all three experimental groups remained within safe physiological limits. This indicates that the HA-coated Mg ZK60 screws do not adversely affect cardiovascular function in experimental rabbits.

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