Toxicological Study of the Magnesium Alloys Mg-(Li)-Y And Mg-Ca-Zn-Mn for Biodegradable Bone Implants

E-poster #22534

Vojtěch Havlas[1, 2], Lucie Vištejnová[3], Filip Hanák[1, 2], Pavel Klein [2]

1. Motol University Hospital, Prague, Czech Republic
2. Second Faculty of Medicine, Charles University, Prague, Czech Republic
3. Charles University, Medical Faculty in Pilsen, Biomedical Center, Pilsen, Czech Republic

Disclosure

- All authors declare, that there are no financial or other conflicts of interest related to this publication.

- All experimental animal procedures were approved by the Advisory Committee for Animal Welfare of the Ministry of Education of the Czech Republic and conducted under the supervision of the Advisory Committee for Animal Welfare of the Faculty of Medicine of the Charles University in Pilsen. The animals received standard care according to the EU Directive 2010/63/EU.

- Research supported by the Ministry of Health of the Czech Republic, grant No. NU20-08-00150.

Introduction

- This study was designed to evaluate the biocompatibility and potential in vivo toxicity of the degradation products of three novel magnesium alloys Mg-3Y (W3), Mg-4Y-4Li (LW44) (both extruded and ECAP forms), and Mg-0,5Ca-1,0Zn-0Mn (extruded) in a rabbit model and compared with well-known commercial magnesium alloy Mg-Y-RE-Zr (WE43).

Why Magnesium?

- Magnesium exhibits unique properties due to the elastic modulus similar to that of the bone, non-toxicity and the ability of human body to remove effectively excessive amount of magnesium. Unlike the permanent implants, biodegradability of magnesium implants opens new possibilities in Orthopedic Surgery.

- So far, many Mg alloys were investigated, among them alloys containing aluminum, zinc, zirconium, yttrium, rare earth elements (REE), etc. Although, possible toxicity connected with the REE is discussed, small implants manufactured from magnesium alloy MAGNEZIX® containing Yttrium, Zirconium and REE are now commercially available.

- We aim to prove that it is not necessary to use REE in magnesium alloys, while advantageous biological and mechanical properties can be preserved, without the potential risk of systemic toxicity.

Materials and Methods

Magnesium alloys used in this study

W3 (Mg-3Y – 3,5 w% Yttrium) ECAP **W3** (Mg-3Y – 3,5 w% Yttrium) Extruded	**Mg-0,5Ca-1,0Zn-0Mn** Extruded
LW44 (Mg-4Y-4Li – 3,5 w% Yttrium, 4,0 w% Li) ECAP **LW44** (Mg-4Y-4Li – 3,5 w% Yttrium, 4,0 w% Li) Extruded	**WE43** (Mg-Y-RE-Zr) (Commercial MAGNEZIX® implants)

Proccessing of the materials

- W3 and LW44 alloys were tested in two structural states achieved by Equal Channel Angular Pressing (ECAP) and extrusion for the purpose of improving their mechanical and corrosion properties.
- Mg-Ca-Zn-Mn alloy was tested only in extruded form.

Important references to previous *in vitro* studies

- No evidence of *in vitro* cytotoxicity of W3 and LW44,alloy was revealed *in vitro.* [1]
- Omitting RE and Zr did not compromise the degradation rate of W3 alloy compared to the WE43, appropriate biocompatibility was preserved. Li addition was found to be biocompatible. [1]
- The overall mechanical performance was the most favorable for W3 alloy (ECAP). [1]
- The in vitro results showed that the W3 alloy (ECAP) exhibits a good combination of ultrafine-grain grain structure, low crystallographic texture, high strength, and comparable corrosion rate compared with the WE43 alloy. [2]

Materials and Methods

Magnesium implants and the surgical procedure

- The screws and pins of W3 (ECAP, extruded), LW44 (ECAP, extruded), and Mg-Ca-Zn-Mn (extruded) alloys (2x10 mm pins and 3x10 mm screws) were machined and used in the expermiment and were compared to well-known commercial alloy Mg-Y-RE-Zr (MAGNEZIX®).
- Two screws and two pins of the same material were implanted into the rabbits femoral shafts (right femurs of the New Zealand White rabbits, females, age > 4 months), N = 3.
- Animals without any implants served as a control.

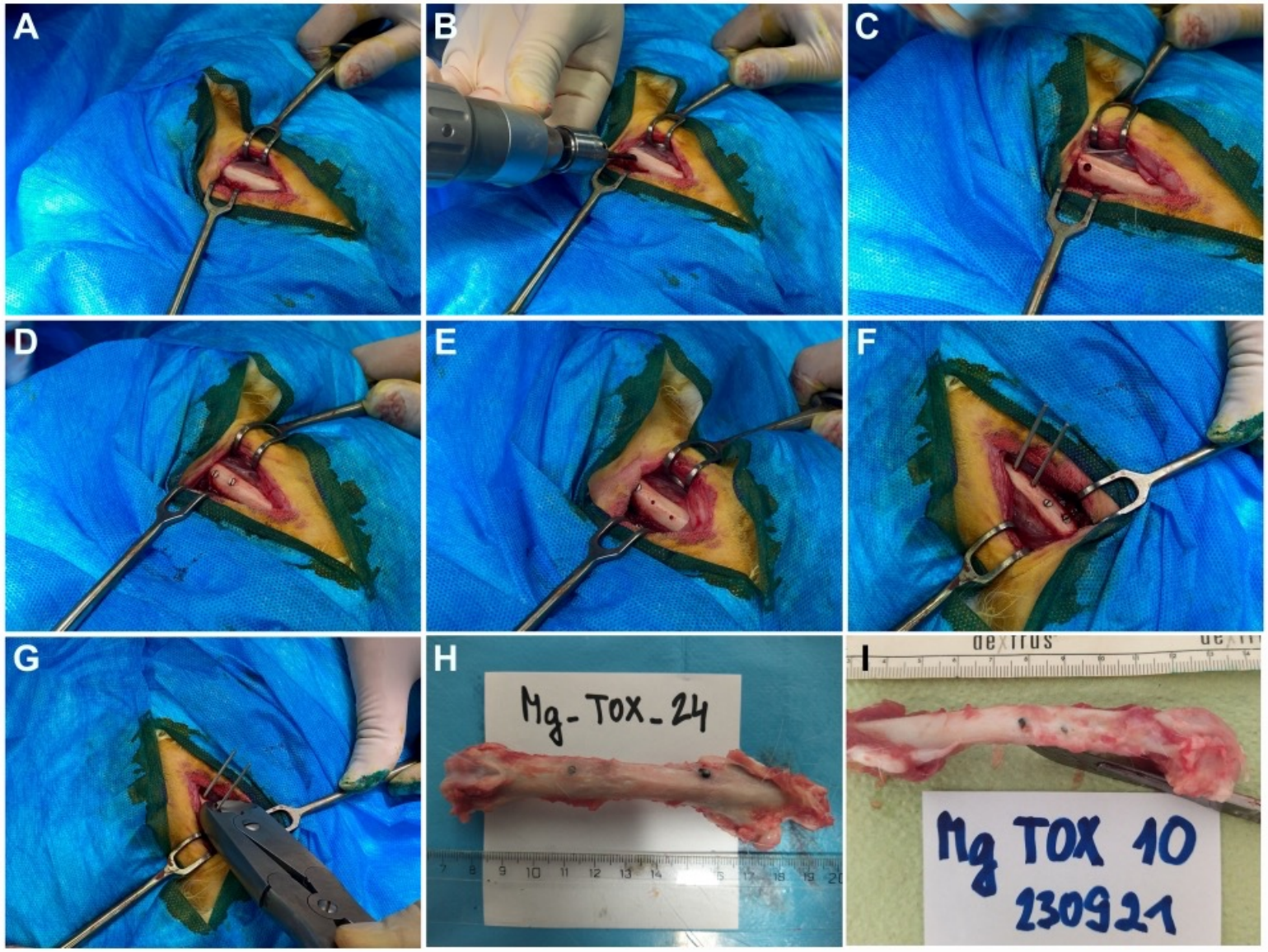
Materials and Methods

Biochemical analysis

- Complete blood count (CBC), liver enzymes (AST, ALT, GGT), bilirubin, albumin, urea, creatinine and levels of Ca, Mg, Li, Zn, Y, Mn elements (contained in the monitored alloys) were tested.
- The levels of Y, Zr and Nd elements (as REE from MAGNEZIX®) were analyzed using ICP-MS (mass spectrometry with inductively coupled plasma).
- Blood samples were collected from the ear central artery on the operation day for a control point, and at 6, 12, 18 and 24 weeks post-surgically in all experimental animals.

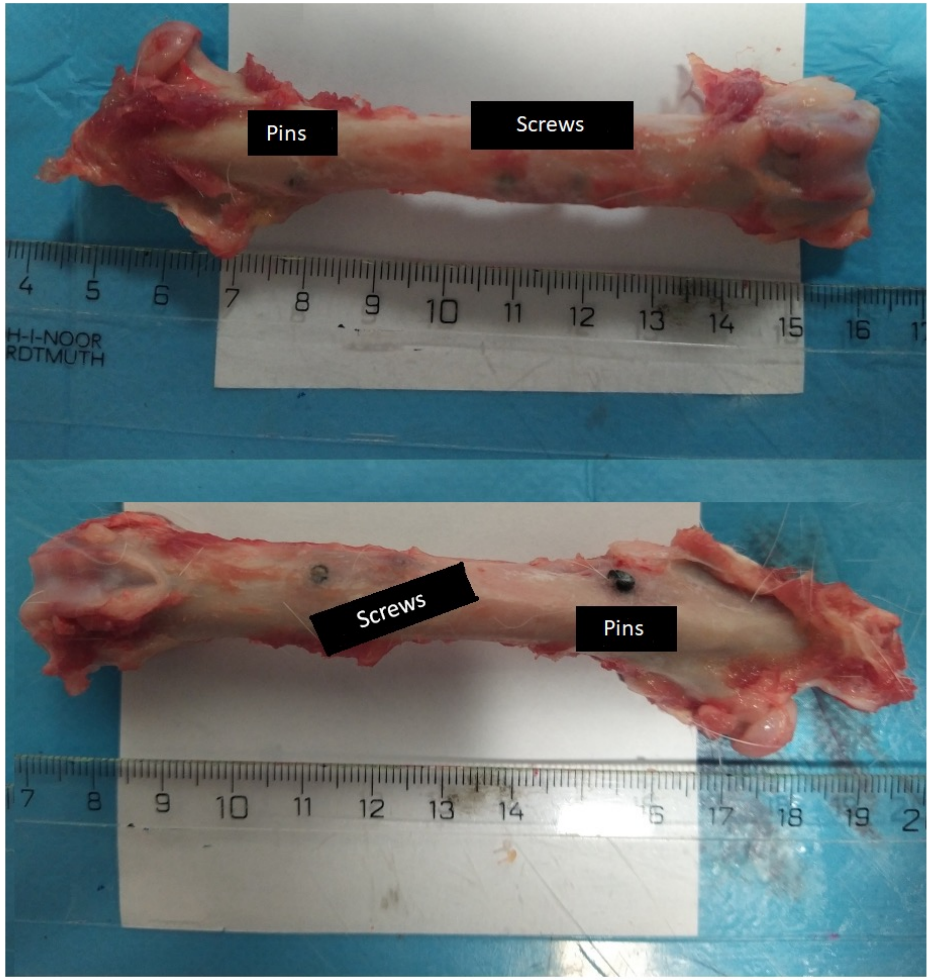
Histological analysis

- The rabbits were euthanized after 24 weeks of implantation to assess potential organ toxicity.
- The tissue samples harvested from the liver, spleen, and kidneys were evaluated.



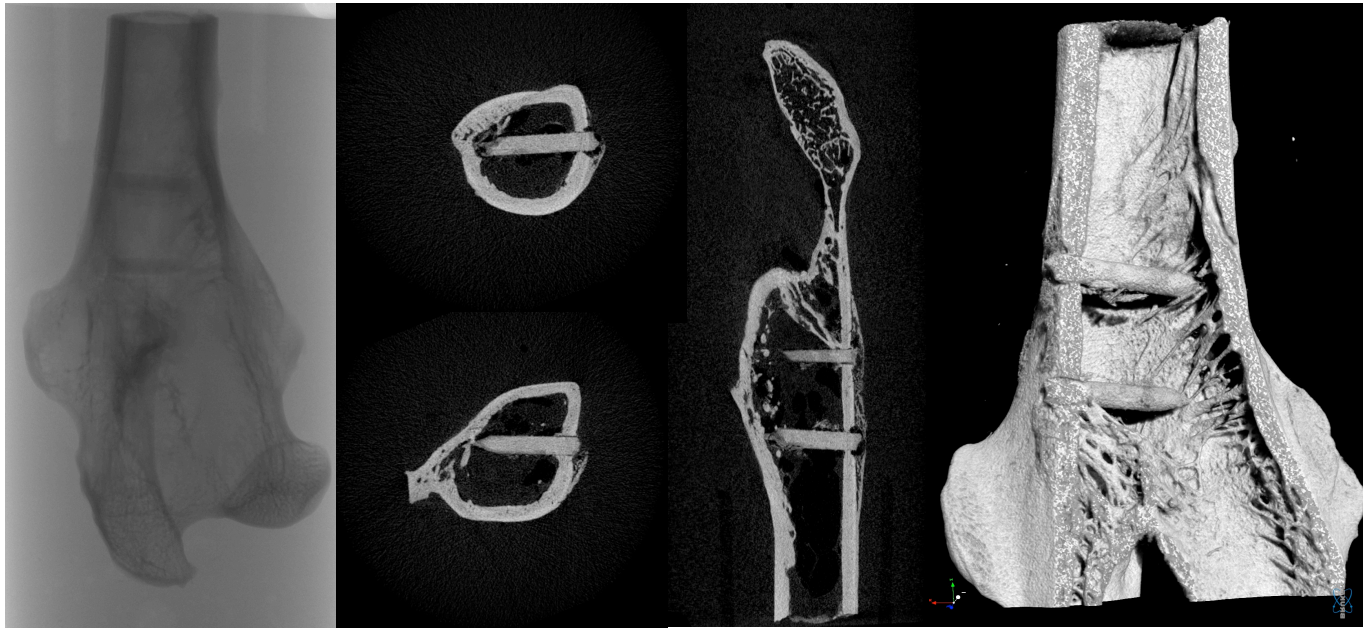
Demonstration of the surgical procedure of implantation magnesium screws and pins.

- A. to G. - photo documentation of the implantation of 2 screws and 2 pins in the rabbit femur.
- H. and I. - example of explanted femurs 6 months after implantation.



Demonstration of femoral explants

Demonstration of explanted femurs 6 months after implantation.



X-ray and CT assessment

Demonstration of the implants positions in the rabbit femur using X-ray and Computer Tomography.

Results

- During the degradation proccess of Mg alloys (W3, LW44, and Mg-Ca-Zn-Mn) *in vivo*, no changes in blood cell count, serum levels of metal ions released from alloys, or any changes in liver enzymes and renal function parameters were detected in the monitored interval 0 – 24 weeks.
- No systemic inflammatory reactions were observed in any of the alloys group *in vivo*.
- In addition, no changes in organ morphology after exposure to all types of alloys after 24 weeks after implantation were observed.
- The in vitro part of the study showed, that the utilization of RE and Zr is not necessary for the biodegradable applications of magnesium alloys and can be easily avoided, while the desirable properties of the material can be maintained.

Conclusion

In summary, our preliminary data indicate that these magnesium-based alloys have good *in vivo* biocompatibility, no systemic inflammatory reactions were observed in any of the animals. In addition, no changes in organ morphology after exposure to all types of alloys after 24 weeks after implantation were observed.

Further research is required to provide evidence of the safety for potential human use.

References

1. Zemková, M., Minárik, P., Jablonská, E., Veselý, J., Bohlen, J., Kubásek, J., ... & Král, R. Ultrafine-Grain Mg-(Li)-Y Alloys for Medical Purposes. *Available at SSRN 4061439*.

2. Zemková, M., Minárik, P., Jablonská, E., Veselý, J., Bohlen, J., Kubásek, J., Lipov, J., Ruml, T., Havlas, V., & Král, R. (2022). Concurrence of High Corrosion Resistance and Strength with Excellent Ductility in Ultrafine-Grained Mg-3Y Alloy. Materials (Basel, Switzerland), 15(21), 7571.