EXPERIMENTAL STUDY

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A new small-animal model for the study of acquired heterotopic ossification after hip surgery

Joris ANTHONISSEN, Christian OSSENDORF, Johanna Lisa HOCK, Ulrike RITZ, Alexander HOFMANN, Pol Maria ROMMENS

University Hospital Mainz, Department of Orthopaedics and Traumatology, Mainz, Rheinland-Pfalz, Germany

Objective: Heterotopic ossification (HO)—the formation of bone in soft tissues—is a frequent problem after surgery of the hip and pelvis, but little is known about its underlying pathogenic mechanisms. It is vital to study the underlying pathogenesis in animal models to develop and evaluate new prophylactic regimens directed against HO. However, previously developed small-animal models for the study of HO imitate neither surgery nor trauma-mechanisms that potentially cause HO. Hence, the goal of this study was to develop a novel small-animal model imitating hip surgery that can reliably produce HO.

Methods: Twenty male Wistar rats were subjected to surgery of the right hip during which the femoral canal was reamed in three steps up to 2 mm, and a muscle lesion was made. Twelve weeks after surgery, the amount of heterotopic bone was assessed using micro-computed tomography.

Results: Eighteen of 20 animals showed HO around the hip 12 weeks after surgery. The amount of heterotopic bone varied from very small particles up to near ankylosis.

Conclusion: A rat model of hip/pelvic surgery that does not use exogenous osteogenic stimulus and can reliably produce HO was developed.

Keywords: Animal model; endoprothesis; heterotopic ossification; hip; pathogenesis.

Heterotopic ossification (HO)—the ectopic formation of bone in soft tissues—is a musculoskeletal disorder that causes pain and reduction in range of motion, often leading to marked impairment of quality of life.[1,2] Acquired HO may occur after virtually any type of musculoskeletal trauma or surgery.[3] Following trauma or orthopaedic surgery, HO formation is commonly observed around hip, knee, shoulder and elbow joints.[4-8] To prevent HO in high-risk patients, indomethacin, celecoxib and radiation are generally accepted as the treatments of choice.[2,5,9] Therapeutic options in HO are limited, and a high recurrence rate is observed. Currently, surgical resection and radiation therapy are the most common treatments to prevent recurrence of HO.[10]

Many animal models have been established to study acquired HO to gain insights into the pathogenic mechanisms underlying this disease.[11-15] However, the most frequently used models (e.g. cutting the Achilles tendon of mice), imitate neither surgery nor trauma mechanisms that potentially cause HO in humans; therefore, their relevance to the condition in humans, and consequently their applicability to humans, remains unclear.

Correspondence: Joris Anthonissen, MD. University Hospital Mainz, Department of Orthopaedics and Traumatology, Mainz, Rheinland-Pfalz, Germany.
Tel: +49 (0) 6131 17 23 59 e-mail: anthonissenjoris@gmail.com

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[16-20] Because there remains no proper treatment or reliable prophylaxis, animal models will remain or even gain importance in the study of HO.[5] To develop a proper prophylactic regimen, the pathogenesis of HO needs to be better understood, and the paucity of relevant models to study the pathogenesis of HO necessitates the development of new animal models.[11] The most suitable model at this time is the Schneider model. However, this model is quite sophisticated and therefore difficult to establish.[21,22] Furthermore, the use of rabbits instead of small animals makes this model more demanding.

The goal of this study was to develop a small-animal model for acquired HO after hip/pelvic surgery. This model was intended to simulate the conditions of hip and pelvic surgery and the pathogenesis of HO thereafter.[15] The need for this type of model has been previously suggested.[11,15,21] Because HO is most often seen after hip surgery, often severely reducing the mobility and quality of life gained by operative treatment of a fracture or arthritis,[8,23,24] it seemed appropriate to modify/simplify the existing Schneider model of HO around the hip and transfer it to rats, which are more amenable to research applications. We hypothesised that this model would produce HO in a reliable fashion, be easy to use, facilitate research into the pathogenesis of HO and make it possible to investigate a prophylactic regimen.

Materials and methods

Twenty male Wistar rats (Charles River, Cologne, Germany) with body weights of 287–365 g were subjected to surgical intervention of the right hip. They were housed in standard cages with a 12-h light–dark cycle at a constant temperature of 21°C, with unlimited access to water and standard dry food pellets. Animal care and management, as well as the surgical protocol and procedures, were approved by the local ethics committee (study number: 23 177-07 / G13-1-047). A licensed experienced veterinarian supervised the animal care and surgery. Anaesthesia was performed using isoflurane (Florane®; Baxter, Deerfield, IL, USA) inhalation for induction, followed by subcutaneous injection of medetomidine (Dorbene®; Pfizer, New York, NY, USA) (0.375 mg/kg), midazolam (Dormicum®; Roche, Basel, Switzerland) (4 mg/kg) and fentanyl (Fentanyl-Janssen®; Johnson and Johnson, New Brunswick, NJ, USA) (0.005 mg/kg) for maintenance. At the end of the procedure, anaesthesia was reversed with subcutaneous atipamezole (Antisedan®; Pfizer, New York, NY, USA) (1 mg/kg). The animals were posi-

Fig. 1. **(a)** Intraoperative image of a rat lying on the left side with the right trochanter major and the incision marked. **(b)** Intraoperative image after skin incision, splitting of the gluteus maximus and exposure of the trochanter major. **(c)** Intraoperative image showing reaming using a drill bit of the femur after entrance to the medullary canal. **(d)** Intraoperative image of the closing of the gluteus maximus after clamping. [Color figures can be viewed in the online issue, which is available at www.aott.org.tr]

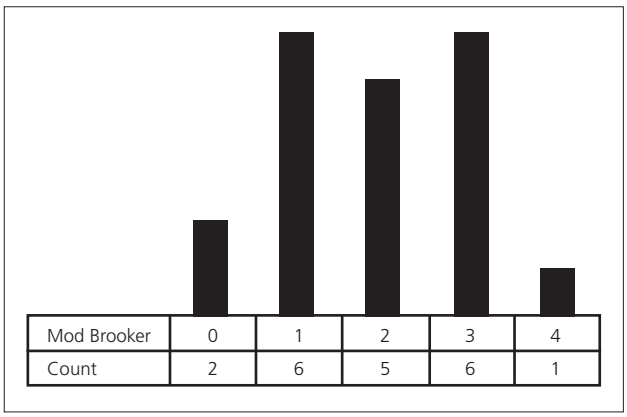

Fig. 2. Grades of HO in different animals. The grade (according to the modified Brooker classification) of HO is shown on the x-axis, and the number of animals is shown on the y-axis.

tioned lying on their left side on the operating table. The right hindquarter was shaved, prepared and draped in a sterile fashion (Fig. 1a). A 1.5-cm incision was made over the greater trochanter, and a sharp dissection was made down to the bone through the gluteus maximus, splitting the muscle in the direction of its fibres (Fig. 1b). After exposing the trochanter, an electric drill with a 1 mm bit was used to gain entrance to the medullary canal just medial to the tip of the greater trochanter. The femoral canal was reamed by hand with incrementally larger reamers up to 2 mm while ensuring that no fracture to the femur occurred (Fig. 1c). The wound was not rinsed to keep the reaming debris in situ within the surgical wound. In addition to the previously outlined procedure, to simulate the muscular trauma that occurs due to retractors, two Kocher clamps were placed across the gluteus maximus and medius muscles (with their points touching each other, creating a 0.5 cm equilateral triangle) for 3 min after femoral reaming. After removal of the clamps, the wound was closed in layers (Fig. 1d). Postoperative analgesia was provided by adding tramadol (0.5 mg/ml) to the drinking water, but no anti-inflammatory agents were used. The animals were permitted to ambulate ad libitum.

After 12 weeks, the animals were euthanised by CO_2 inhalation. The right hip with the proximal half of the

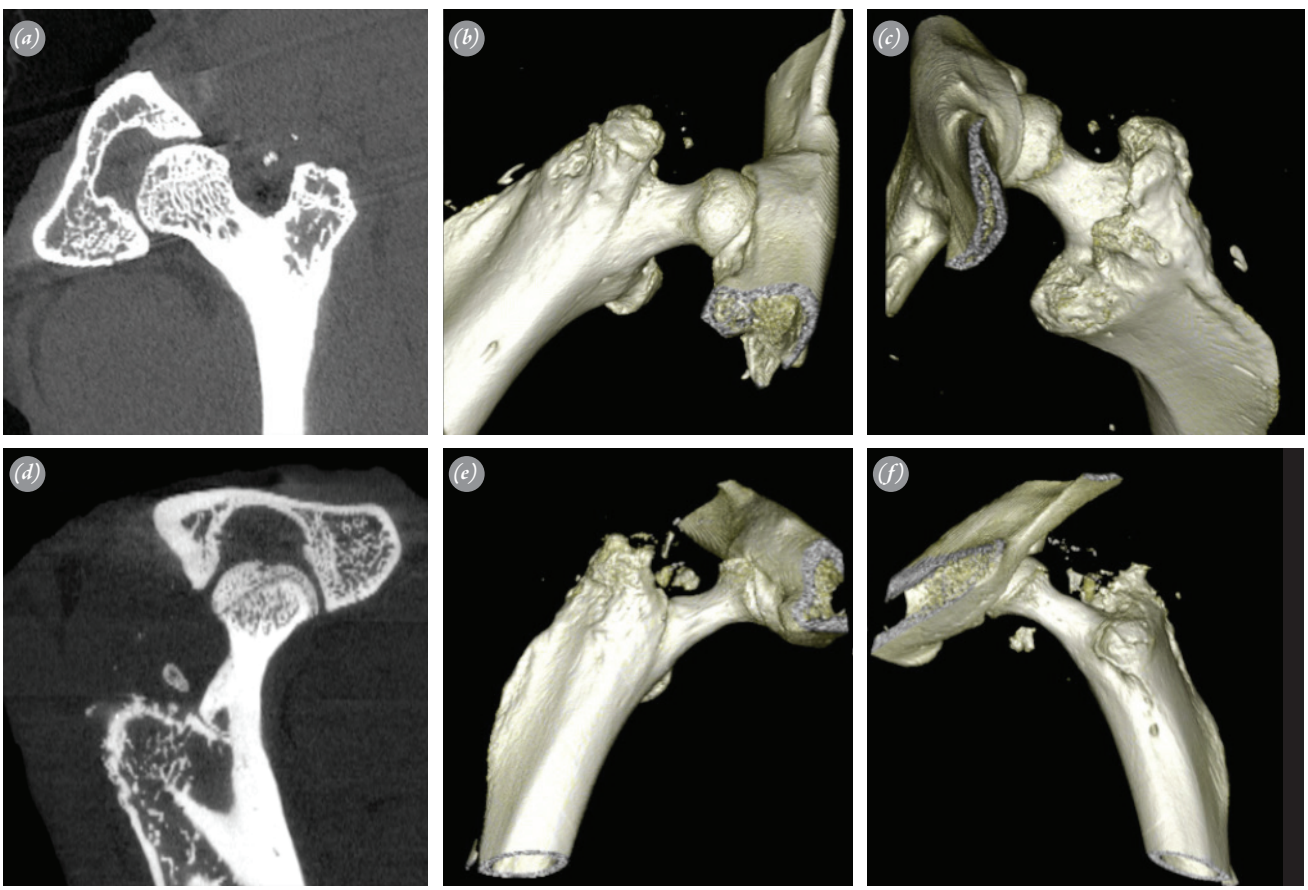

Fig. 3. μCT images of a rat with grade 1 HO. **(a)** Native ✂CT image of the hip of a rat with grade 1 HO; **(b and c)** 3D reconstructions of μCT images of the same rat. μCT images of a rat with grade 2 HO. **(d)** Native μCT image of the hip of a rat with grade 2 HO; **(e and f)** 3D reconstructions of μCT images of the same rat. [Color figures can be viewed in the online issue, which is available at www.aott.org.tr]

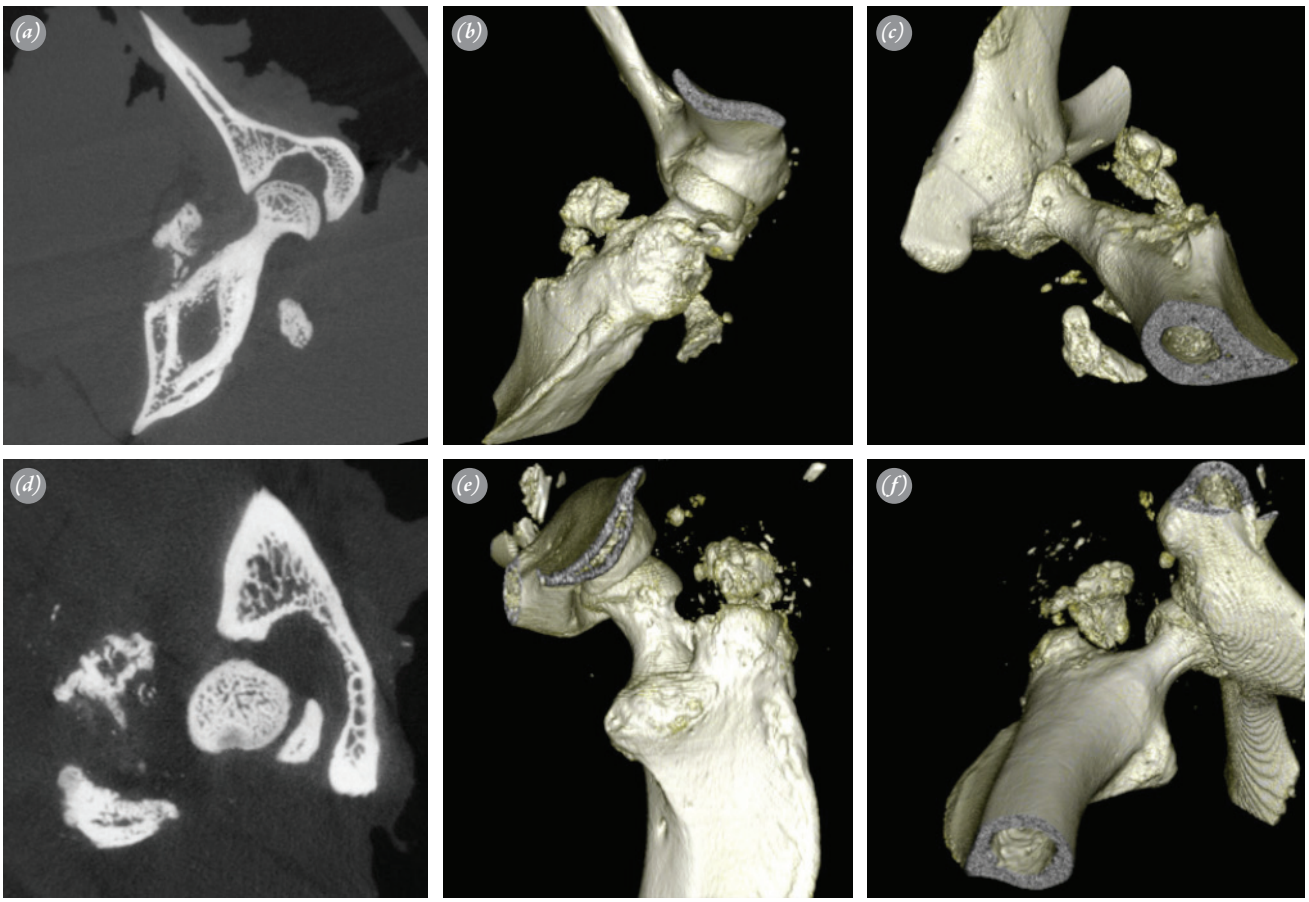

Fig. 4. μCT images of a rat with grade 3 HO. **(a)** Native μCT image of the hip of a rat with grade 3 HO; **(b and c)** 3D reconstructions of μCT images of the same rat (large ossicles can be seen on each side of the femoral neck). μCT images of a rat with grade 4 HO. **(d)** Native μCT image of the hip of a rat with grade 4 HO; **(e and f)** 3D reconstructions of μCT images of the same rat. [Color figures can be viewed in the online issue, which is available at www.aott.org.tr]

femur and approximately half of the pelvic bone with the surrounding soft tissue were resected and removed, fixed in paraformaldehyde and subjected to micro-computed tomography (μCT) analysis by using a μCT-40 scanner (Scanco Medical AG, Bassersdorf, Switzerland). Data were analysed by using a modified Brooker classification system. A grade (0–V) was attributed to the observed heterotopic bone around the hip. A grade of 0 was given when no HO was found, I when very small particles of heterotopic bone (all measuring <1 mm) were seen, II when 1–2 mm particles were observed, III when >2-mm particles were observed, IV for near ankylosis (leaving a <3 mm space between the major fragments—i.e. trochanter–heterotopic bone and heterotopic bone–acetabulum) and V for total ankylosis of the femur to the pelvis.

Results

All animals survived the procedure; they began to ambulate immediately postoperatively and were able to return to normal mobility after 3 days. μCT of the prepared specimen showed no HO in two animals. In all other animals, HO of different grades was seen (Fig. 2): six animals showed grade I (Fig. 3a-c), five showed grade II (Fig. 3d-f), six showed grade III (Fig. 4a-c), and one showed grade IV (Fig. 4d-f); no total ankylosis (grade V) was observed.

Discussion

The aim of this study was to develop a novel animal model that could reliably produce HO without the addition of an exogenous osteogenic stimulus. In our model at 12 weeks, eighteen of twenty rats showed HO, as evidenced by μCT. This finding demonstrated the ability of the model to produce HO in a reliable fashion without exogenous stimuli. An earlier attempt by Toom et al. to develop a similar model in rats was unable to demonstrate the development of HO without the addition of a bone morphogenetic protein-containing matrix. Their result was probably because of the limited postoperative interval (3 weeks) to euthanasia.[25]

Two homologous HO models without exogenous stimuli have previously been developed: the aforementioned Schneider model in rabbits and the extremity blast amputation model of Tannous, the latter being an excellent model of the rather rare condition of HO after extremity blast amputation.[15,21] The Schneider model is challenging because it involves the use of rabbits (which have to be intubated and relaxed to ream the femur, and housing is more difficult and expensive), rather than small animals.

The formation of HO as represented by the Schneider model is probably due to osteogenic molecules from the bone marrow that are transferred to damaged muscle in which mesodermal stem cells differentiate into bone cells.[26-28] Further research is needed to determine which molecules induce HO and how to influence this without compromising fracture healing or bony growth on the surface of an implanted prosthesis.

In conclusion, a hip-surgery rat model that does not require the use of exogenous osteogenic stimuli and can reliably produce HO after 12 weeks was developed. This model warrants further consideration for the study of the pathogenic processes underlying HO.

Conflics of Interest: No conflicts declared.

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