Acta Orthop. Belg., 2014, **80**, 2-10

REVIEW ARTICLE

Animal models for acquired heterotopic ossification

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Heterotopic ossification (HO), the ectopic formation of bone in soft tissues, is a relevant musculoskeletal disorder that, by reduction of range of motion, may lead to significant impairment of quality of live. HO can either be acquired or hereditary. Acquired HO is seen most often after hip prosthetic surgery and pelvic trauma. In contrast, hereditary HO is commonly observed in the axial skeleton, but can affect every joint. Substantial effort has been directed towards understanding the pathophysiology and towards finding both, effective prophylactic and therapeutic treatments. Every improvement of the understanding of the pathophysiologic changes underlying HO as well as the rationale of prophylactic and therapeutic treatment regimens in the end, is based on the study of appropriate animal models. Although intriguing models of 'genetic' HO have been developed recently, their relevance to acquired HO remains questionable.

As there is still neither proper treatment nor reliable prophylaxis, animal models will remain important in the study of HO. Currently, there are 6 different animal models regularly used for the study of acquired HO. Some of these models can reflect a merely particular part of the disease. Hence, selection of the appropriate animal model for the study of HO is exceedingly important. The present paper reviews the history and major features of the different animal models of acquired HO, and reveals some of the insights gained through the study of animal models ; important biochemical and pathophysiological key features are highlighted. Clinical studies have proved indometacine, celecoxib and radiation therapy to be effective in reducing the occurrence of HO, but not always be able to prevent it.

INTRODUCTION

The first description of heterotopic ossification (HO) goes back to a scientific contribution by Patin in children suffering from 'myositis ossificans progressiva' published in 1692 (*68*). Later, a more distinct description of HO was provided by Riedel *et al*, as well as by Dejerine and Ceilier in 1883 and 1918, respectively (*16,24,75*). Although HO is usually defined as a new formation of trabecular bone including bone marrow in soft-tissues, where bone usually does not occur (*4,18*), there is no consensus on the name, definition and classification of HO (*64,103*).

HO is no trivial disease ; the formation of heterotopic bone can lead to a limitation of the range of motion and may have serious consequences for the quality of life of patients (*103*).

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No benefits or funds were received in support of this study.

Two types of HO are distinguished : genetic and acquired (*87*). Many authors suggest a similar pathogenesis of genetically caused and acquired HO (*1, 41,55,86,90*). Genetically caused HO consists mainly of two disorders, fibrodysplasia ossificans progressiva (FOP) (*43*) and progressive osseous heteroplasia (POH). Vast deposits of heterotopic bone characterize these two entities with progressive accumulation around several joints. This process eventually leads to severe disability and early death due to pneumonia (*56*). A wealth of research has recently been done on the pathophysiology of FOP (*42,110, 111*). The genetic regulation of some bone morphogenetic proteins (BMP) and its inhibitory factors were proven to be impaired, and suggested as a major effector underlying genetically caused HO (*42,110,111*). In contrast, the acquired form usually is either precipitated by trauma or has a neurogenic cause (*87*). HO may occur after virtually any type of musculoskeletal trauma. The most common site for the formation of HO is the pelvic bone after open-reduction internal-fixation (ORIF) for acetabular fracture, followed by the hip after total hip arthroplasty (THA) (*2,4,8,27,77,78,95*) ; but also after orthopaedic procedures of the knee, shoulder or elbow, and fractures, respectively. Even after joint dislocation or direct soft tissue trauma, the formation of HO was observed (*22,30,36*). In contrast to the hip and pelvis, abdominal incisions, wounds (specially war amputation wounds), the kidneys and the gastrointestinal tracts are less commonly encountered sites of posttraumatic HO (*20,32,37,65,71,72*). The other form of acquired HO (i.e. HO with neurogenic cause) occurs after injury to the nervous system, usually after traumatic brain injury or spinal cord injury (*20,67*). Bone formation following neurologic injury tends to form in para-articular sites. The most commonly affected joint in neurogenic HO is the hip, followed by shoulder and elbow. Neurogenic HO rarely occurs around the knee (*21,38*).

The incidence of HO after THA ranges between 16 and 53%, (*103*) but only a minority of the patients becomes symptomatic (3-7%) (*4,20*). In contrast, the incidence of HO following neurologic trauma is reported to be 10-30% (*92*). In the prevention of HO after THA, in patients with high risk, indomethacin, celecoxibs and radiation are generally accepted as the treatment of choice (*4,76,103*). In the prevention of HO after neurologic trauma, much controversy exists on the use of range-of-motion exercises (*44, 53*). Therapeutic options in HO are limited and a high recurrence rate is observed. Currently the most common treatment is surgical resection and radiation therapy to prevent recurrence of HO (*92*).

As the pathophysiology of HO remains unclear, the limited current available prophylactic and therapeutic interventions appear to be neither sophisticated nor always effective. Practically every better understanding of the pathophysiologic changes underlying HO and the rationale of prophylactic and therapeutic treatment regimens owe their origins to the study of animal models. Animal models reflecting pathophysiology, prevention and treatment will thus play an important role in the future (*4*). Currently 6 major animal models are used. Here, we review their history and major features including the respective advantages and disadvantages. The most important insights gained through the study of these animal models are highlighted as well.

ANIMAL MODELS

Achilles tenotomy model

The occurrence of HO in humans after achilles tenotomy was first described by Jones in 1932 (*39*). He observed painful ossifications in achilles tendons 10 years or more after achilles tenotomy. This observation presumably ended in the development of an achilles tenotomy model in rats in 1953 (*9*). Buck described a simple mid-way division of the achilles tendon using a sharp razor blade after skin incision and blunt dissection. Thereafter, only the skin was closed, i.e. no adaptation of the tendon ends using sutures. In this model, HO could be shown in all specimens by the end of a three-month-period (*81*).

In 1969, Salah was able to show that it isn't necessary to divide the achilles tendon. He observed that the squeezing of the tendon with an artery forceps also led to HO. Furthermore, he demonstrated that, when two ligatures are placed around the achilles tendon, the segment between the ligatures is gradually converted into a large ossicle (*82*). He

also proved that no HO is formed when the calf muscles are denervated. Therefore the pull of the muscles on the achilles tendon seemed to be of great relevance. In 1983, McClure wrote an article that is judged as the standard work on the Achilles tenotomy model. He applied the achilles surgery described by Buck to mice and found that ectopic bone developed in 60% of animals after 5 weeks and in 100% after10 weeks (*57*). The advantages of this model are its relative simplicity and excellent predictability. However, the molecular mechanisms of HO induced by Achilles tenotomy are poorly understood, and the relevance to clinical conditions is unclear since ectopic bone formation in the achilles tendon is a rare condition in humans (*40*). Recently this model is often used in rats and mice to research different preventive strategies to reduce the occurrence of HO formation (*12,51,83,114,116*). The validity of these finding in comparison to humans is however largely unclear. Also in the pathogenetic research of HO this model is used with the same limitations (*40,52*).

Immobilisation-manipulation (Michelsson) model

In 1980, Michelsson *et al* could show that the repeated and intensified mobilization of the knee joint in rabbits causes formation of HO in (rabbit) quadriceps muscles (*58*). Michelsson developed a model that was characterized by rabbit's knee immobilisation for 5 weeks with a plastic splint and elastic bands. During this period, the splint was removed each day and the knee was passively and intensively mobilized for 5 minutes through the full range of motion. After this 5-week period, the splint was removed and animals were allowed to move freely. During this experiment, HO was seen radiographically in all animals at the end of the 5-week period. Further growth of HO could be demonstrated after the 5 first weeks. In a second publication in 1994, Michelson *et al* showed, by placing a membrane between the quadriceps and the femur, that isolation of bone from muscles prevented the development of experimental callus-like HO (*59*). Therefore, Hardy criticized in 1997 the finding of Michelson arguing that it is not HO that is seen in his model but a dystrophic calcification (*33*). In a reaction on this Michelson stated that bone developed in the vastus intermedius muscle where in normal circumstances no bone is found and that therefore his findings should be called HO. This discussion highlights the problems that arise from the lack of a consensus on the definition of HO. Since his publication in 1980 several authors have used this model to study the development and prevention of HO in rabbits (*5,60,98,104,105*). The first sign of osteoblastic activity was seen in the periosteum, and the new bone was often formed in continuity with the periosteum. Interestingly, early changes in prostaglandins preceded bone formation consistent with the hypothesis that inflammation is the basis of the heterotopic bone formation in that process (*98*). Although it seems that the interaction between the periosteum and the necrotic muscle are necessary for the formation of HO, since the introduction of a plastic membrane between bone and muscle prevents bone formation (*59*), the precise inductive stimulus has not been identified in this model. Therefore its relevance to human HO remains unclear.

Implantation / injection models

The most commonly used animal models in the research of possible therapy and prevention of HO involve the surgical implantation of BMP containing matrices or injection of BMP containing substances at heterotopic sites (*40*). These models have been employed for over 80 years. In 1938, Levander was able to induce the formation of cartilage and bone in 23% of animals by injecting alcoholic extracts of autologous bone into the rectus femoris muscle of rabbits (*3,48,49*). Bertelsen (1940) proved that alcoholic extracts of bone marrow (83%) were superior to extracts of cortex, epyphisis and periosteum (48%) (*6*). Lacroix (1945) obtained bone with hematopoietic marrow with extracts of epiphyses of new-born rabbits (*45*). He suggested that the hypothetically inducing substance be called 'osteogenin'. Lagos (1946) and Heinen (1949) challenged these findings by stating that the injection of alcohol alone had the same effect as injecting an alcoholic bone extract (*35,46*). They therefore denied the existence of a specific osteogenic sub-

stance. In 1957 Danis found that after heterotopic transplantation of bone marrow, bone was formed at the site of heterotopic implantation (*15*). Chalmers, Burnwell and Friedenstein confirmed these findings in 1959, 1964 and 1966, respectively (*10,11,19*). In 1965 Urist showed in different animals that samples of diaphyseal decalcified bone implanted in a pouch in the belly of a muscle gave rise to new bone formation by what he called auto-induction (*100*). This model of implanting demineralised bone matrix into soft tissue was rapidly adopted by others and is still used to research the induction of bone / HO in soft tissue and how to prevent the formation of HO (*11,14,17,54,61,97,117*). Reddi (1972) characterised this bone formation extensively and found that it mirrors the normal process of in vivo cartilage and bone formation (*74*). Urist and collaborators identified in 1979 the active component in the bone extracts used in these early experiments and named it bone morphogenetic protein (BMP) (*101, 102*). Wozney *et al* were able to repeat this experiment using partially purified BMP proteins (*108*). These experiments proved the existence of a specific osteogenic substance that Lacroix called osteogenin (*45*). Currently, the most widely used approach is BMP-matrigel implantation at heterotopic sides (*25*). Many modifications/variations of this method have been used in different species under different conditions to assess the pathogenesis and prevention of HO (*31,34,47,62,88,89,106*). In recent experiments, transfection of BMP coding genes into animal soft tissue is used to get a better knowledge of the pathogenesis of HO and bone formation in general. In these experiments adenoviruses, retroviral viruses, and plasmid particles containing BMP-genes (mostly BMP 2 or 4) are used to induce HO (*26,66,69,70,109*). Inhibitors of bone formation as Noggin and Gremlin are also injected in this way to evaluate their function (*23,99,115*).

Another intriguing version of this model researches the osteoinductive ability of certain biomaterials, such as micro-porous calcium phosphate ceramic particles, that do not release BMP or other known osteogenic factors (63). The mechanism of osteoinduction by such biomaterials is not currently clear, although the geometry of the material is thought to play an important role (*40,112*).

Generally, heterotopic implantation / injection models are straightforward, reliable, and mechanistically relevant to human HO. However, certain limitations do exist : (1) they are artificial systems that may create non-physiologically high local concentrations of osteogenic factors at implanted sites leading to effects not relevant to the human disorder, (2) the implantation is a local event and thus has limited ability to mimic the potential effects of the involvement of systemic factors.

Hip surgery (Schneider) model

Schneider *et al* described a model to simulate the pathogenesis of HO after hip arthroplasty in 1998 (*84*). In this model, male New Zealand rabbits were treated similarly to human hip arthroplasty using a standard approach anterolateral to the hip. The left hip of the animals underwent muscle injury by clamping to produce ischemia of gluteus maximus and medius muscles. The right hip underwent no muscle injury and served as a control. The medullary canal of the femur was opened and reamed comparable to the implantation of a hip prosthesis. The reaming-debris was left in situ. This straightforward model was reported to produce HO with high reliability in 17 of 18 animals with no significant difference in amounts of bone formation between muscle injury side and control side.

Rumi successfully used this model in 2005 to assess the optimal timing of prophylactic preoperative radiation and to identify the origin of osteoprogenitor cells responsible for HO (*79,80*).

Toom *et al* used this model as described by Schneider in rats to research the role of osteoprogenitor cells from the femoral canal (*96*). In their research, the induction of HO (without BMP-2 implants) was not successful. Only cartilage was found in the examined samples. This is probably because the animals were sacrificed at 3 and 21 days already. In contrast, as described in the original publication, Schneider *et al* studied the animals radiographically after 1 to 7 months and histologically after 7 months only (*84*). Using this hip surgery model, Tannous *et al* proved that the formation of bone in HO is endochondral. In this experiment the first 21 days only cartilage was

formed and only later this cartilage would calcify and reorganise to lamellar bone (*94*). This model is a mechanism-based model, reflecting a known cause of HO in humans. It seems therefore a suited model to study the formation of HO after THA. Whether this model is also relevant to other forms and causes of HO remains questionable.

Direct trauma models

Traumatic muscle injury can lead to bone formation in soft tissue of humans. McCarthy and Sundaram (2005) termed this type of bone formation 'myositis ossificans circumscripta' (*56*). They described it as a self-limiting disorder in which an osseous mass develops close to bones and joints, mostly initiated by a trauma and typically seen in patients 15-30 years of age.

Efforts to establish trauma-induced models had only limited success. Back in 1904, Haga and Fujimura reported to have evoked ossification in traumatized animal tissue (*29*). Gruber challenged this finding in 1913 by stating that Haga and Fujimura did not reveal their method nor their exact results (*28*). In line with his criticism, Gruber self failed to induce ossification in rabbit thigh muscle using single hammer strikes. In 1926 Stone was unable to detect ossification in dogs after striking the anterior surface of the thigh during partial or complete relaxation (*91*). Using a mini version of a pile driver, Zaccalini and Urist (1964) were not able to induce HO in rabbit thigh (*113*) In an intriguing study, Collins *et al* (1965) induced bone formation by stripping the periosteum of the thigh bone and damaging the overlying muscle (*13*). Ossification was enhanced by repeated blunt trauma over the thigh after injury. Walton *et al* (1983) reported the induction of intramembranous ossification within scar tissue in sheep following blunt trauma of the thigh (*107*). The induction of ossification was only successful in 16.6% of traumatized thighs, further, intramembranous and not endochondral ossification was the histological feature within scar tissue. More recently Tannous *et al* induced heterotopic ossification in rat in an extremity blast amputation model (*93*). In this model extremity amputation was produced through detonation of an explosive while protecting the animal proximal to the specified amputation level. This model was able to produce HO with a good reliability (4/4 hind limb, 1/5 fore limb) especially in hind limb amputations.

Based on the presented reports, we conclude that most of the models described here do not seem to be suffciently reliable to be routinely used. It remains unclear whether the formation of bone as described in these studies can be defined as HO. The recent model of Tannous *et al* might be of relevance to study the formation of HO after blast amputation in war setting, as HO in the residual limbs of combat-related amputees has been reported in up to 63% of patients (*71,93*).

Irritant injection model

Heinen *et al* reported the induction of HO in rabbits by injection of 40 % ethanol (*35*). As the injection of various irritant substances into muscles was reported to lead to the formation of HO, many trials to identify further substances inducing HO were undertaken. Selle and Urist for example reported that acid-alcohol could induce HO in a small percent of animals, while injections of calcium chloride led to calcification in soft tissue only (*85*). Others used a mixture of phosphatase glycerophosphate and alginate gel (*7*). In contrast, in their search to find a specific osteogenetic substance, alcoholic subtracts of bone were used. In most of these publications, pure alcohol is used as a control, but no HO formation was found (*3,49,50,73*). The insufficient repeatability and questionable clinical relevance of these models grossly limits their potential use.

CONCLUSION

Currently 6 animal models are known to mimic HO. Most enable to reflect some forms, particular aspects, or only distinct varieties of the human condition. The questionable reliability and ambiguous clinical relevance of most of the models complicate their use in the search for prophylactic and therapeutic treatments for HO. Results of studies with models with unknown relevance to the condition in human should therefore be carefully examined, as

conclusions and interpolation of the respective results may not always be comparable to the human condition. It is therefore the authors opinion that, in order to get a full understanding of the pathogenesis of HO, more mechanism-based models as the hip-surgery model and the extremity blast amputation model are needed. In the mean time, it remains important to choose the right model that fits the question asked in the study of HO and be aware of its limitations when drawing conclusions towards the human condition.

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