



The pathogenesis of heterotopic ossification after traumatic brain injury. A review of current literature

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Neurogenic heterotopic ossification (NHO), mostly defined as a benign process of formation of bone outside the skeletal system, after traumatic brain injury (TBI) is a musculoskeletal disorder that causes pain and reduces the range of motion, often leading to marked impairment of quality of life. The pathogenic factors that link the brain and bone and cause the formation of heterotopic bone are largely unknown. This article will try to summarize the current literature on the pathogenesis of NHO and accelerated fracture healing after TBI.

The heterotopic formation of bone after TBI seems to be induced by a complex interplay between local and systemic factors. For all different forms of HO, the same three conditions are required for the formation of ectopic bone: The presence of osteoprogenitor cells, a permissive environment, and a stimulating factor. The osteoprogenitor cells are thought to be of mesenchymal origin, however recent research suggests a possible neural origin. The permissive environment is created mainly by reactions to hypoxia and both local and sensory nerve inflammation. Many possible inducing factors have been described; the endogenic route is thought to be the most dominant in the stimulation of HO formation after TBI.

The pathogenesis of NHO remains largely unknown, recent research, however, has discovered interesting topics for further research and new possible targets in the prevention of NHO.

Keywords: heterotopic ossification; traumatic brain injury; pathogenesis.

INTRODUCTION

Neurogenic heterotopic ossification (NHO), mostly defined as a benign process of formation of bone outside the skeletal system, after traumatic brain injury (TBI) or spinal cord injury is a musculoskeletal disorder that causes pain and reduces the range of motion of joints, often leading to a marked impairment of quality of life (22,45,46). The incidence of HO after TBI is reported to be about 11% (79,88,93). It is known that as the severity of the TBI increases so does the incidence and volume of HO. Additional trauma to the hip or pelvis further increases the risk for the development of HO (22,36,79). For the prevention of NHO after TBI, the same protocols are used as in the prophylaxis of HO after total hip arthroplasty. NSAIDs, bisphosphonates, and radiotherapy are

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commonly used (8,24,87,108). Therapeutic options in HO are limited and a high recurrence rate is observed (35). Currently, surgical resection and peri-operative radiotherapy to prevent recurrence is the most common treatment (19). The pathophysiology of NHO after TBI remains largely unknown. Interestingly it has previously been observed that TBI does not only induce the formation of heterotopic ossification, but that around fractures it causes an abundant callus formation and appears to cause a more rapid union as well (15,62). Although there is an increasing understanding of both the molecular basis and metabolic regulation of bone healing, our knowledge of what controls the rate of fracture healing is incomplete (6). Whether the observed changes genuinely represent accelerated fracture healing or are a form of local heterotopic ossification remains unclear (42).

A lot of effort has been directed towards finding a link between brain injury and new bone formation (93). It was postulated that the research on acquired HO and the understanding of the osteogenesis after TBI could not only help to prevent and treat this disease but would greatly enhance the current tools for treating non-union and segmental bone defects if one would be able to harness this phenomenon (51). This article will try to summarize the current literature on the pathogenesis of NHO after TBI, with a special focus on new insights and possible therapeutic targets.

PATHOGENESIS

The heterotopic formation of bone after TBI seems to be induced by a complex interplay between local and systemic factors. Using animal models it was proven that the formation of heterotopic bone once started follows the same pathways as the formation of osteochondral bone (92). The exact initiating mechanism, however, remains poorly understood. The result of these largely unknown interactions is a differentiation of pluripotent stem cells into bone-forming osteoblasts and an increased activity of these osteoblasts (91,96).

For all different forms of HO, the same three conditions are required for the formation of ectopic bone: The presence of osteoprogenitor cells, a

permissive environment and a stimulating factor (67). This review will use these three conditions as the main structure to summarize the current understanding of the pathogenesis of NHO.

The osteoprogenitor cells

The presence of osteoprogenitor cells is one of the conditions required for HO to form. There is some evidence for their dormant state in most connective tissues, for example in muscle, fascia and most organs (93).

Mesenchymal stem cells (MSCs) in skeletal muscle

MSCs are pluripotent and are able to form bone, cartilage, fat, tendon, muscle and nerve tissues after a triggering signal. Mineralized nodules, which later lead to bone formation, have been proven to arise from vimentin-positive spindle-shaped cells. According to morphological, immunohistochemical and mRNA profiles as well as their capacity for multilineage differentiation, they belong to the group of MSCs (83,100).

Historically it was assumed that the progenitor cells were muscle-derived as injection of bone marrow-derived concentrates could induce heterotopic ossification in muscle tissue (20,30). Some more recent studies pointed towards a derivation of these cells from the interstitial cells between the muscle fibers (55,101). Recent research on war-traumatized muscle tissue confirmed the presence in the skeletal muscle of progenitor cells with many analogies (Cell surface markers, morphology, osteogenic potential, multipotency, and osteogenic gene expression) to bone marrow-derived MSCs (44).

Endoneurial progenitors in nerve endings

Recently however this belief of muscular origin was challenged. Lazard et al. found that within 24 hours after the induction of HO through an injection of the quadriceps of mice with Bone morphogenetic protein 2 (BMP-2) producing cells, osteoblast-specific transcription factors appeared in cells of the endoneurium followed by their coordinate disappearance from the nerve at 48 hours. They reappeared in blood at 48 hours after

induction to disappear again from the circulation at approximately 3 to 4 days by extravasation into the site of new bone formation. They, therefore, concluded that the endoneurial progenitors are the major osteogenic precursors that are used for HO. In their opinion, this might explain the increased formation of HO after TBI or SCI. This raises the question of whether endoneurial cells play a major role in the enhanced callus formation after TBI as well (51).

Others

Apart from the above mention two main possible origins of the progenitor cells in NHO some other possible origins have been mentioned in the literature. These include bone marrow (69), circulation (89), and the interesting idea that fully differentiated endothelial cells that can transition into MSCs might play a major role in the development of HO (58,59).

The permissive environment

The second condition is a permissive environment, which should provide an appropriate scaffold to support the growth of bone and the blood supply during bone formation. The concurring local injury in TBI patients is thought to contribute significantly towards creating an environment that is permissive to the formation of bone (46). Some clinical studies, however, described HO in critically ill patients without local injury on the site of HO formation. This fact seems to contradict the importance of a permissive environment created by local injury. It is suggested however that local micro traumata (i.e. forced mobilization) or the direct effect of an altered neural stimulation may in some cases be sufficient to create a permissive environment (65,67,80).

Many contributing factors have been investigated : Tissue hypoxia, alkalosis, salt precipitation, and electrolyte disturbances, as well as changes in local sympathetic nerve activity, immobilization or forcible mobilization after prolonged immobilization and local imbalance between PTH and Calcitonin concentrations.

Hypoxia

Hypoxia seems to be one of the main factors contributing to creating a permissive environment (29). Several studies suggest that low oxygen tension critically influences chondrocyte differentiation by accelerating the growth of mesenchymal stem cells and promoting their commitment to the chondrocyte lineage, in part by up-regulating chondrocyte-specific gene expression under the control of hypoxia-inducible factor 1 alpha (HIF-1 α) (66). In an animal model study in rats, it was proven that inducing hypoxia resulted in an increased volume and incidence of HO (5).

Brown adipocytes are suggested to be another possible hypoxia inducing factor. Olmsted-Davis et al. used an animal model to demonstrate the presence of brown adipocytes after local injection of BMP-2 (66). Later the same research group was able to demonstrate the presence of brown adipocytes in human tissue samples of heterotopic ossification after combat-related trauma. These brown adipocytes were atypical and seemed to have a unique function in the development of HO. Therefore they were suggested as an important possible target for the prevention of HO (75).

Neo-angiogenesis

During HO formation, which follows the pathway of endochondral ossification (92), avascular cartilage tissue must be replaced by highly vascularized bone. This hypoxic stress-induced transformation is mediated by angiogenesis stimulators such as vascular endothelial growth factor (VEGF) or platelet-derived growth factor (PDGF). This ultimately initiates the differentiation of MSC into osteoblasts. Histological studies have already shown that osteoblasts and their progenitor cells always develop accompanied by endothelial cells in new blood vessels, the target of the newly formed vessels being hypoxic tissue (4,27,106).

In a mouse model with BMP-2-induced HO, Dilling et al. observed that new blood vessels were formed even before the onset of cartilage. As early as 48 hours after BMP-2 injection, brown adipocytes accumulated in the affected tissue, which on the one hand created a hypoxic milieu and on the other

hand produced and released VEGF, both inducing angiogenesis (28).

Considering these results, neovascularization seems to be essential for ectopic bone formation. The exact role it plays in this process is not yet fully understood, but inhibition of angiogenesis has successfully reduced the formation of HO in animal models (110). Especially VEGF and HIF-1 α appear to be possible targets in the prevention and therapy of HO (68,98).

PDGF

Angiogenesis and osteogenesis are thought to be closely related during ectopic bone formation as they share important mediators, such as PDGF (an essential growth factor for angiogenesis) (99). Werner et al. investigated the effect of inhibiting PDGF expression on ectopic bone formation in a murine Achilles tendon tenotomy model. They injected imatinib, a PDGF inhibitor, into the test mice and were able to detect a reduction of the HO volume formed by 85% compared to untreated control animals. The authors hypothesized that the formation of new blood vessels was needed to allow MSC to reach their target site to form ectopic bone (99).

VEGF and HIF-1 α

Once tissue hypoxia is established, the affected cells are stimulated to produce HIF-1 α . As a result, VEGF is released, which eventually promotes angiogenesis and the associated oxygen and nutrient supply to the affected tissue (61,82). It is well recognized that the formation of new blood vessels into cartilage tissue is essential for physiological endochondral bone formation and healing. Many authors assume that VEGF and HIF-1 α play an important role in this process (23,27,94). The formation of new blood vessels prior to the formation of ectopic bone has already been described many times in the specialist literature. However, VEGF appears to be an essential coordinator between cartilage resorption, angiogenesis, and the formation of mature bone during endochondral ossification (17,39).

Ueno et al. using a rabbit transplant model investigated the importance of VEGF during the

formation of HO. They transplanted periosteum of tibial origin into the suprahyoidal musculature of the animals, thereby establishing a connection between the graft and the periosteum of the mandible. From the 14th day post-transplantation, VEGF-positive chondrocytes in the suprahyoid musculature were detected by immunohistochemistry. According to the authors, an invasion of blood vessels into the VEGF-positive cartilage areas followed before the ectopic bone was formed. The osteoblasts also showed a positive VEGF expression on immunohistochemistry (94).

Recent research has uncovered a significant role of HIF-1 α , a regulator of VEGF, in the coupling between hypoxia, angiogenesis and bone formation (2,14,31). In 2013, Zimmermann et al. Reported that echinomycin, a HIF-1 α inhibitor, significantly reduced HO after Achilles tendon tenotomy in mice (110).

Neuroinflammation and the Blood-nerve Barrier

Studies on the neural involvement in the formation of HO have highlighted the role of neuroinflammation and the blood-nerve Barrier (BNB) (26).

It was postulated that the proximity of large nerves makes certain regions more prone to the formation of HO (i.e. The hip with the proximity of the sciatic nerve, the elbow with the ulnar nerve) (21). It is thought that stretching these nerves weakens the BNB (32). This could also explain the high incidence of HO after amputation and blast injury (3,57,85). It is thought that a TBI further increases the expression of Substance P (sP), calcitonin gene-related protein (CGRP) and metalloprotease 9 (MMP9) locally and hereby making the local environment even for permissive to the formation of HO (38,81).

sP and CGRP

It is thought that local injury (or the expression of BMP-2) causes neuroinflammation through the release of sP and CGRP (77). It was demonstrated that an elevated expression of BMP-2 induced a release of sP and CGRP, leading to the recruitment and degeneration of mast cells (74). In the same study, it was proven that blocking these mast cells led to an

inhibition of the HO formation (74). Interestingly in the research of the genetic form of HO, sP was also found to be a critical mediator (47).

CGRP was also found to be able to induce chondrogenic differentiation of progenitor cells after spinal cord injury in a mouse model study (76).

MMP9

Another possible factor in the opening of the BNB during the formation of HO seems to be the expression of MMP9, (one of the few enzymes linked to opening the BNB by degrading the extracellular matrix) associated with the activation of IL-6 (54,63,90). It was even suggested that MMP9 could be used as a marker of an active process of HO formation (71).

The stimulating factors

The endogenic route

By treating human osteoblasts with serum from patients that suffered from a TBI, a significant increase in proliferation could be observed (10,50). An increase in proliferation of MSCs and an increased expression of alkaline phosphatase was observed as well (11,16). These findings indicate the existence of humeral factors that increase the differentiation and proliferation of osteoblasts as well as mineralization after TBI. A lot of possible inducing factors have been described (72,93). As a lot of the factors that are found to be elevated after TBI are seen as well in the healing of fractures and the normal development of bone, the main question remains which of these factors are responsible for the formation of HO after TBI.

BMP

BMPs were discovered by Urist during his attempts to induce bone in muscle-tissue (95). BMP-2, 4 and 9 are recognized as the most important factors for regulating the formation of bone. As they strongly promote osteoblast differentiation and bone induction (49,52,102,103). Several studies have shown that BMP-2 and 4 can reliably induce bone (49,52,101). The level of BMP and its receptors is up-regulated after TBI and muscle around the hip show an increased level of BMP-2 and 4 (41,53,78). As

stated above BMP-2 seems to induce the production of brown adipose tissue and stimulate sensory neurons to release sP and CGRP (66,74,75). The fact that BMPs can induce the formation of bone in soft tissue is indisputable since their discovery by Urist. It remains however difficult to assess their role in the physiological process of the formation of HO as in most animal models, HO is induced using a high concentration of BMPs (60).

Thrombin

TBI causes abnormal blood coagulation and thrombin is released immediately after its occurrence (73,104). Osteoblasts express the protease-activated receptor-1, through which thrombin mediates many cellular responses (1). Thrombin is released as well on at the place of local injury (97). These combined make thrombin able to induce osteogenic precursor cells to migrate to the injury site and promote the proliferation of well-differentiated osteoblast (48). It is thought that thrombin might have a role in enhanced fracture healing as well (70).

IL-6

High levels of interleukin-6 (IL-6) are observed during the inflammatory phase of bone healing and IL-6 is released after TBI (7,18). Some authors, therefore, suggest it might play an important role in the initial phase of fracture healing and the formation of HO after TBI (10,33,40).

Direct neurogenic effects

A direct neurogenic effect is gaining more interest as the loss of sympathetic control of bone homeostasis could alter bone formation and density (13,64). It is thought however that these effects occur mainly after mild-TBI and result in a net bone loss (9,12,13,84,107). The effect of TBI on the formation of HO seems therefore dose-related. The mechanisms underlying this observation are largely unknown and a definition of mild TBI is lacking (43).

Cerebrospinal fluid (CSF)

A route through the CSF that causes the formation of HO after TBI has been suggested. In most of these

studies, CSF of TBI patients was added to a cell culture or the presence of known factors that can induce bone were measured in the CSF after TBI (25,37). There is however no proof that these factors in the CSF can get to remote locations to induce the formation of HO other than through an endogenous transport after injury of the Blood-brain barrier. Factors suggested to act through the CSF-route are Leptin, Melatonin and Substance P (56,105).

Fracture healing and TBI

It is known that fractures heal faster with a more abundant callus formation in patients with concomitant TBI (34,109). Some authors suggest that this is due to the addition of neurogenic progenitor cells that support the callus formation and create some sort of neurogenic HO around the fracture (16,51,62,86). Others suggest that TBI just raises the osteogenic capacity in the body both orthotopic (around a fracture) and heterotopic (72). Therefore, there might be no separate entity called neurogenic HO (37,91). There is no consensus on the validity of these theories, but it is generally assumed that the enhanced bone healing and the increased formation of HO share the same principles and both reflect the enhanced osteogenesis after TBI (43).

CONCLUSION

The pathogenesis of HO after TBI remains not fully explained. However recent studies confirmed the release of osteogenic factors from the brain in the systemic circulation. The new insights into the inflammation of sensory nerves and the potential neurogenic origin of osteoprogenitor cells provide interesting focuses for further research. Lastly, the enhanced angiogenesis during the formation of HO provides a possible therapy target and warrants further research as well.

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