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Review article

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Heterotopic ossification as a side effect of the use of recombinant human bone morphogenetic proteins

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Abstract

Introduction Heterotopic ossification (HO), also known as myositis ossification, paraosteoarthropathy, or heterotopic calcification, among others, is a common pathological condition that refers to ectopic bone formation in soft tissues. Although the molecular mechanism of HO is not fully understood, it is believed that signaling of bone morphogenetic proteins (BMPs) plays a key role in the overall process of HO. Today, recombinant human BMP-2 (rhBMP-2) and recombinant human BMP-7 (rhBMP-7) have been already actively used in clinical practice in the treatment of bone defects. However, despite the positive sides of using rhBMPs, there are a number of side effects, one of which is HO. **Purpose** In this study, we demonstrate cases of HO following the use of rhBMPs in both clinical and preclinical studies and make an attempt to explain the relationship between the signaling pathways of BMPs and the HO process, as well as the possibilities of preventing and treating the HO process. **Materials and methods** PubMed, Embase, the Cochrane Database, and Google Scholar were comprehensively searched for original articles, literature reviews, case reports, and meta-analyses demonstrating a causal relationship between therapeutic rhBMPs and HO as a complication. **Results** This review analyzes the potential for therapeutic use of rhBMPs in neurosurgery and traumatology and orthopedics, demonstrated by both clinical and preclinical studies. In particular, the studies confirm that ectopic bone formation is one of the side effects following administration of rhBMPs. Moreover, the molecular mechanisms of the HO process were highlighted, and the possibilities of modern methods of prevention and treatment of HO were discussed. **Conclusion** According to the FDA safety database for rhBMPs, the rates of adverse effects related to HO range from 1 % to 10 %. However, to date, the clinical use of rhBMPs is justified, especially when there are no alternative substitutes for bone grafting.

Keywords: heterotopic ossification, recombinant, bone morphogenetic proteins, mechanism, treatment

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INTRODUCTION

Heterotypic ossification (HO), also known as myositis ossificans, paraosteoartopathy, or heterotopic calcification, is the formation of ectopic lamellar bone in soft tissues [1]. In acquired HO, the pathological process is a complication due to injuries of the central nervous system (CNS), burn damage to soft tissue, injuries or surgical interventions [1]. The knowledge of the molecular mechanisms that lead to HO and of the progenitor cells involved in this process is still limited. Different populations of progenitor cells may be possible precursors to HO development. *In vivo* studies indicate that progenitor cells may vary and depend on the HO subtype. The studies demonstrate that endothelial cells (ECs), mesenchymal stem cells (MSCs), and pericytes that are present in striated muscle, tendons, and connective tissue, or even circulating stem/progenitor cells may be a source of HO development [1, 2]. It is also known that trauma, which leads to a local inflammatory response, activates certain signaling pathways that are directly involved in the development of HO. Moreover, recent *in vitro* and *in vivo* studies have proven the role of immune system cells, especially monocytes/macrophages, in the early HO stages [2, 3]. In particular, those studies support the importance of monocytes/macrophages in the induction of neurogenic and genetic HO types. Activated monocytes/macrophages express osteoinductive signaling factors in HO (Fig. 1). Thus, the presence of the cells reflects increased secretion of growth factors, cytokines/chemokines that stimulate HO, such as interleukins (IL-6 and IL-10), transforming growth factor beta-1 (TGF-β1), neurotrophin 3 (NT3), and bone morphogenetic proteins (BMPs) (BMP-2, 4 and 7) [4, 5].

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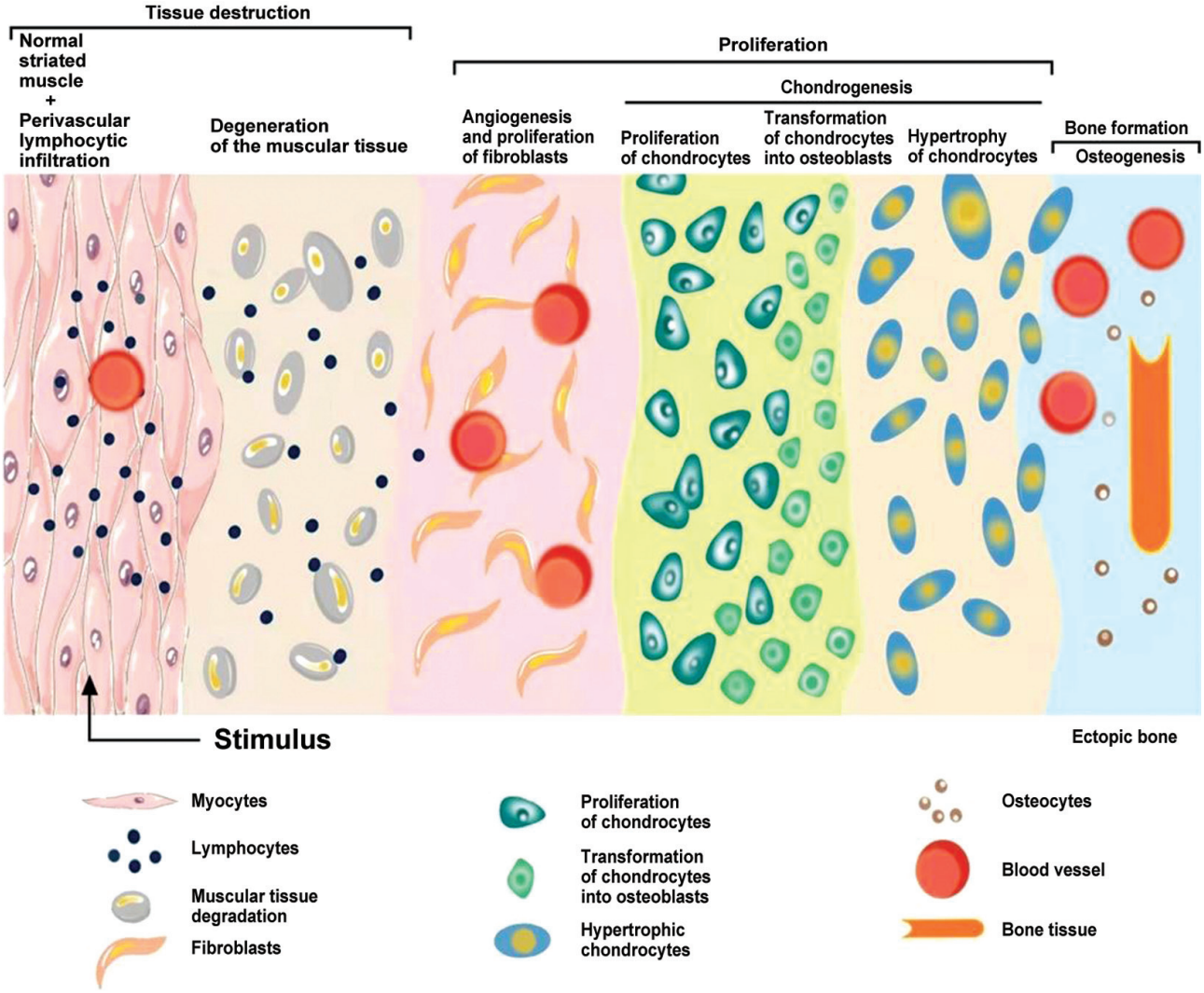

Fig. 1 Schematic presentation of HO stages. The formation of ectopic bone is divided into three stages: 1) under the influence of various stimuli or factors, a large number of perivascular lymphocytes may be accumulated due to overcoming the vascular barrier and their migration to the early HO region within the striated muscle what is accompanied by the destruction of the connective tissue structure; 2) proliferation of connective tissue cells and destruction of the striated muscle with the release of inflammatory mediators, which in turn stimulate fibroplasia and angiogenesis; and 3) synthesis of a number of growth factors that induce osteogenesis by formation and differentiation of mesenchymal stem cells (MSCs) into chondrocytes and osteoblasts in the local inflammatory microenvironment. The differentiation processes results in the formation of ectopic bone in the area of tissue damage. (authors' drawing)

The most potent osteoinductive growth factors are multifunctional cytokines belonging to the TGF-β superfamily, and namely BMPs. BMPs have a significant osteoinductive effect on various stages of the bone healing process after injury, such as the inflammatory response, angiogenesis, soft and hard callus formation, and bone remodeling [6]. The use of recombinant human bone morphogenetic proteins (rhBMPs) has been shown in preclinical and clinical studies to promote de novo bone formation, accelerate bone recovery time after injury, and prevent nonunion or delayed union [7]. In particular, one rationale for their use in open fractures is based on the concept that accelerating bone regeneration and preventing nonunion reduces the complication rate and the need for reoperations [8]. Apart from the fact that BMPs have osteoinductive properties, there are scientific studies with evidence that BMPs may stimulate angiogenesis [7]. However, despite the significant positive effects of rhBMPs, their widespread use in practical medicine is limited due to a number of disadvantages such as their rapid degradation, high production cost, the need for high doses, osteolysis, and HO. In this paper, we demonstrate reports on the cases of HO after the use of rhBMPs in both clinical and preclinical studies. Moreover, we attempted to explain the relationship between BMPs signaling pathways and the HO process. We also show the possibilities of preventing HO after rhBMPs application.

MATERIAL AND METHODS

We conducted a comprehensive search for original studies, literature reviews, case reports and meta-analyses that show the relationship between rhBMPs used for therapeutic aims and HO as a complication of their application. PubMed, Embase, Cochrane Database and Google Scholar were searched for corresponding studies in the period of August and September 2021. The key words were *bone morphogenic proteins or recombinant bone morphogenic proteins, growth factors or TGF-β family, heterotopic ossification or ectopic bone, or ossificating myositis, or pathomorphology, or complications, and clinical studies or preclinical studies, or therapy and prevention, or molecular mechanism or side effect*. Reference lists of the studies found were also searched for related works. The diagram of systematic review is presented in Figure 2.

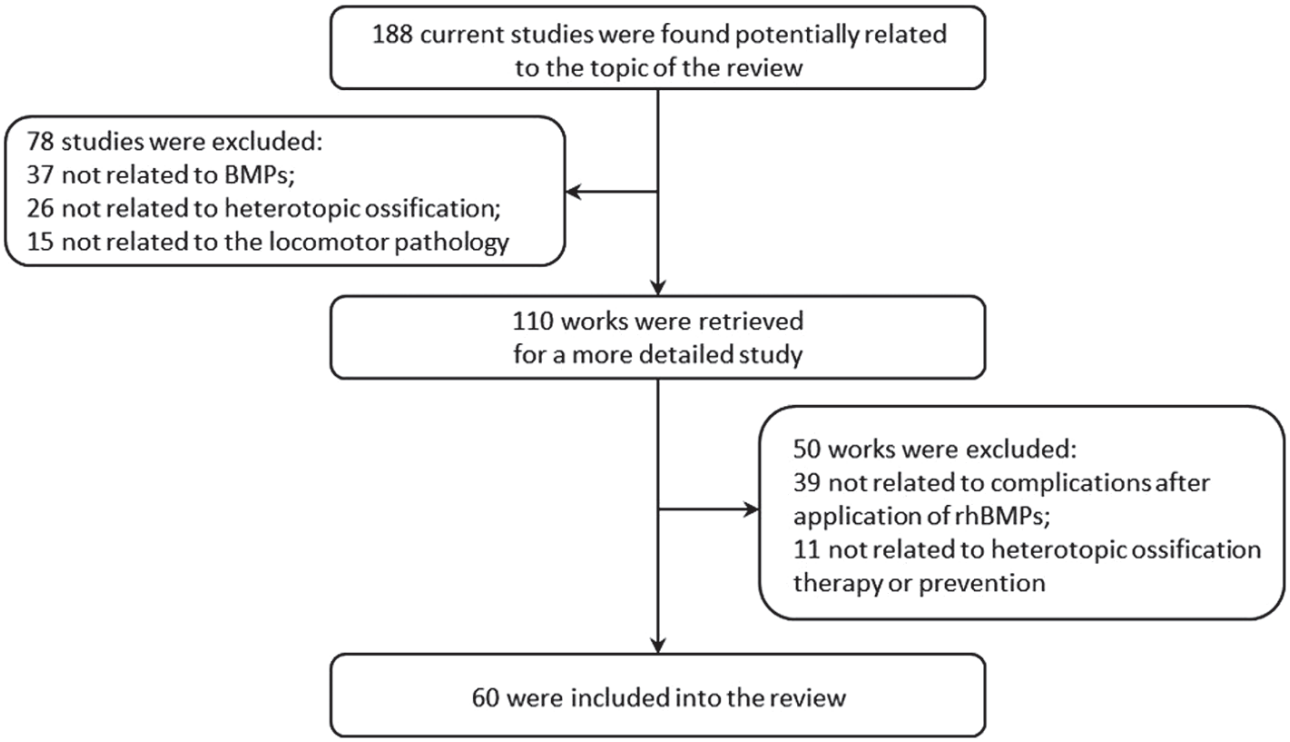

Fig. 2 The flow chart of a systematic review

RESULTS AND DISCUSSION

1. BMPs and HO

The local inflammatory process leads to the migration, reproduction and differentiation of many cell types, including progenitor cells [2]. However, the molecular mechanism underlying the process of HO types has not been fully understood. It is believed that progenitor cell populations, inductive growth factors, and a favorable environment may contribute to HO progression. It is well known that progenitor cell differentiation along the chondrogenic pathway and endochondral ossification is facilitated by various factors, including BMPs signaling and transcription factors [8]. According to the theory of Kan et al., there might be one conservative mechanism involved simultaneously in many HO types [9]. Moreover, recent evidence suggests a central role of BMPs in the pathogenesis of HO, along with other growth and transcription factors involved in the cross-linking between downstream signaling pathways of BMPs [10].

BMPs are a member of the TGF-β superfamily, that are essential for embryonic development and maintenance of tissue homeostasis. BMP-induced bone formation occurs not only under normal conditions, but also in pathological processes. Increased BMPs signaling caused by a defect in the autoregulatory loop was confirmed in lymphocytes from HO patients [11]. Moreover, high levels of BMP-2 and BMP-7 were found in the blood serum of patients after CNS injuries [12, 13]. Also, the expression of BMP-2, 4, 7, and 9 was significantly increased in animal models of HO caused by spinal cord injury [14]. BMP-2, 4, 7 and 9 should be considered as main BMPs candidates in the

HO pathophysiology. BMPs ligands signal through a tetrameric complex of type I and type II receptors. Seven type I receptors (Activin receptor-like kinase 1-7 (ALK1-7)) have been identified, among which BMPs preferentially bind to ALK 1, 2, 3, and 6. Once the ligands bind to type II bone morphogenetic protein receptors (type II activin A receptor (ACVR2A or Act 2A) and type II activin 2B receptor (ACVR2B or Act 2B)), they transphosphorylate each other with activation of type I receptor kinase, where subsequently activated type I receptor kinases phosphorylate R-Smads (Smad 1, Smad 5 and Smad 8) to induce the formation of a complex with Smad 4. Subsequently, the complex moves to the cell nucleus, where it activates the expression of the target gene (Fig. 3) [6, 15]. Thus, it is widely accepted that dysregulation of BMPs signaling leads to HO.

2. Dosage and carriers

It has been suggested that high doses of administered rhBMPs may lead to ectopic bone formation [16–18]. In all clinical trials conducted, the dosage of rhBMP varies greatly. It is believed that HO may be induced by supraphysiological doses of rhBMPs, which should be used in humans for effective treatment to overcome the short half-life of BMPs and their rapid clearance *in vivo* (~6–7 min) [19]. Thus, Boraiah et al. reported a high risk of HO in the treatment of complex tibial fractures using high doses of rhBMP-2 [20]. Clinical doses of rhBMP-2 may range from 0.1 to 0.5 mg/kg body weight, although doses as high as 1 mg/kg body weight have also been reported [21]. The doses of rhBMPs used in clinical trials presented in Table 1 ranged from 1.4 to 12 mg [16–18, 22–28]. The results of some of the studies provide evidence that a positive outcome is achieved with low doses of rhBMPs and suggest that this is the reason of the lower HO complication rate. However, the limitations of the current studies are significant and do not allow unambiguous conclusions about the dose-dependent complication of ectopic bone formation by using rhBMPs.

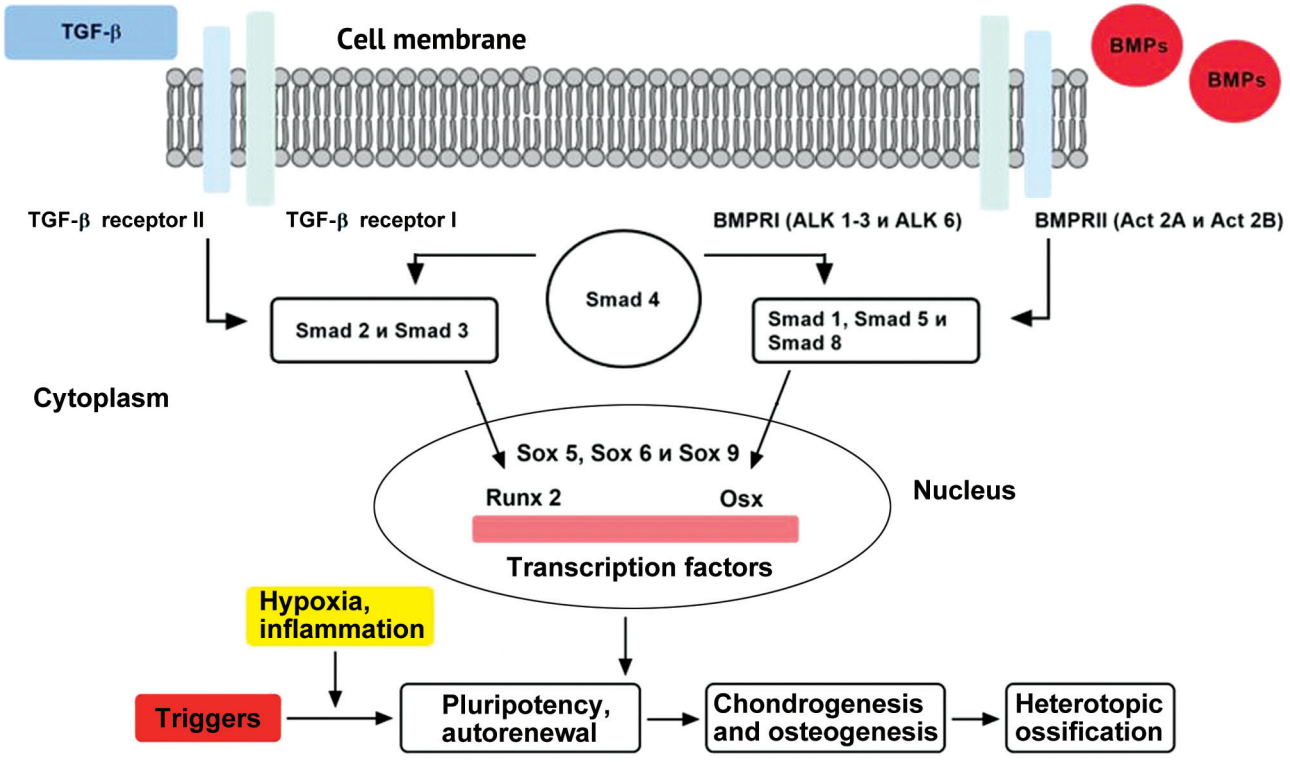

Fig. 3 Regulation of the BMP / TGF-β / Smad signaling pathway in heterotopic ossification. The BMP / TGF-β signaling pathway is initiated by a tetrameric complex of type I and type II receptors. Once ligands bind to type II receptors, they transphosphorylate each other and activate type I receptor kinases, followed by phosphorylation of the R-Smads (Smad 1, Smad 2, Smad 3, Smad 5, and Smad 8) complex with Smad4. The complexes move to the nucleus, where they recruit transcription factors to regulate the expression of target genes. The BMP signaling pathway is involved in the overall development of heterotopic ossification, including inflammation and hypoxia. BMPs, bone morphogenetic proteins; TGF-β, transforming growth factor-β; ALK, activin receptor-like kinase; Sox, SRY-boxing transcription factor; BMPR I, type I bone morphogenetic protein receptor; BMPR II, type II bone morphogenetic protein receptor; Runx 2, Runt-associated transcription factor 2; Osx, osterix; Act, actin (authors' drawing)

The current standard carrier is an absorbable collagen sponge that delivers rhBMPs via physical absorption of the protein onto the implant material. However, this is limited by the "explosive" release and leakage of proteins beyond the intended site of implantation [29]. Therefore, these supraphysiological doses of rhBMPs led to HO, mentioned earlier [22–24]. It has recently been shown that new carriers based on polyelectrolyte complexes (PECs) incorporating heparin on the surface of alginate microbeads fabricated using the layer-by-layer polyelectrolyte self-assembly principle deliver rhBMP-2 in a controlled manner and significantly reduce protein release [30]. In their work of Wang et al. showed that a heparin-based polyelectrolyte complex as a carrier material for rhBMP-2 demonstrated the ability to minimize the formation of HO by reducing the effective dose in an animal model of spinal fusion [31]. The results of that study indicate that heparin-functionalized alginate microbeads may be a new option in finding an effective rhBMP-2 carrier with fewer complications and lower cost. From a molecular point of view, several endogenous growth factors, including BMPs, are known to exist in the extracellular matrix compartment through sequestration (or strong non-covalent anchorage) of negatively charged, highly sulfated heparin-like glycosaminoglycans. Thus, the biological activity of BMPs is preserved. Following this model of storage and regulation, many in vitro and *in vivo* studies demonstrated that key glycosaminoglycan molecules such as heparin and heparan sulfate may be used to modulate the biological activity of various exogenous growth factors, including BMPs [32, 33].

Thus, minimally invasive methods of applying rhBMPs, their low concentrations, and the use of modern carriers for BMPs can reduce the risk of ectopic bone formation.

Table 1

Studies on the use of recombinant bone morphogenetic proteins (rhBMPs) in clinical practice that show development of heterotopic ossification (HO)

rhBMPs	Procedure	Study design	Number of patients, n	Dose, mg	Carrier	Success, n (%)	Heterotopic ossification, n (%)	Reference number
rhBMP-2	PLIF	Randomized prospective multi-centre study	34	4–8	Absorbable collagen sponge	31 (92)	26 (75)	16
rhBMP-2	TLIF и PLIF	Prospective observational study	23	4.2	Absorbable collagen sponge	23 (100)	5 (21)	22
rhBMP-2	PLIF	Prospective observational study	17	12	Absorbable collagen sponge	17 (100)	1 (6)	23
rhBMP-2	PLIF	Prospective observational study	30	1.4	Absorbable collagen sponge	29 (97)	2 (7)	24
rhBMP-2	TLIF	Retrospective cohort study	933	1.0	INFUSE (Medtronic)	863 (92,5)	125 (13,5)	25
rhBMP-2	Management of the acetabular defect in revision hip arthroplasty	Case report	1	2.8	–	1 (100)	1 (100)	17
rhBMP-2	Femoral head osteonecrosis	Retrospective cohort study	46	4	–	38 (83,3)	8 (17,3)	18
rhBMP-7	Management of long-bone nonunion	Retrospective cohort study	84	3.3	Bovine collagen type I	68 (80,9)	15 (17,8)	26
rhBMP-2 и rhBMP-7	Treatment of acute fractures and delayed union	Case series	4	According to product protocol	Infuse and OP-1	4 (100)	4 (100)	27
rhBMP-7	Management of distal humeral nonunion	Case report	1	3.5	OP-1	1 (100 %)	1 (100 %)	28

Notes PLIF – posterior lumbar interbody fusion; TLIF – transforaminal lumbar interbody fusion; rhBMP – recombinant human bone morphogenetic protein; OP-1 – osteogenic protein-1.

3. Clinical studies

RhBMP-7 and rhBMP-2, also known by their trade names as osteogenic protein-1 (OP-1) and INFUSE, are approved by the US Food and Drug Administration (FDA) and have found their way into clinical practice. One of the side effects associated with the use of rhBMP-2 and rhBMP-7 is the formation of ectopic bone [34]. It appears that a variety of non-osteoblast cells undergo osteogenic programming when exposed to BMPs, including myoblasts, adipocytes, and fibroblasts. It was estimated that ectopic bone formation in clinical trials was almost six times more frequent than in the control group (the allograft group without the use of rhBMPs). Computed tomography showed ectopic bone formation in 70.1 % of patients treated with rhBMP-2 compared to 12.9 % of patients who were not treated with rhBMP-2 [35]. Thus, during spinal surgery, the rhBMPs may spread outside the implants in the epidural space and lead to the formation of ectopic bone in the soft tissues, followed by compression of the peripheral nerve roots. Thereby, the rate of postoperative sciatica may reach 14.0 % compared to 3.0 % in control groups [35]. Moreover, the manufacturers of rhBMP-2 and rhBMP-7 recommend that some precautions be taken when handling the rhBMP/Absorbable Collagen Sponge product to minimize extravasation of BMPs. According to the commercial product instructions of the Medtronic company for rhBMP-2 and rhBMP-7, it is essential to avoid "irrigation or aspiration near the implant" and "excessive squeezing of the soaked sponge" [34]. Table 1 lists clinical studies on the use of rhBMP-2 and rhBMP-7 in orthopedic cases that were complicated by HO [16–18, 22–28].

4. Standard methods of HO prevention and treatment

Treatment of HO can be divided into actions aimed primarily at preventing the disease, and actions aimed at HO elimination. In the case of prevention, pharmacological treatment and radiation therapy have been used. In pharmacological prophylaxis, cyclooxygenase-2 (Cox2) inhibitors and non-steroidal anti-inflammatory drugs (NSAIDs) are most commonly used, both groups of drugs act on pro-inflammatory prostaglandins [36]. Traditional NSAIDs such as aspirin, ibuprofen and indomethacin inhibit the formation of both physiological and inflammatory prostaglandins. Cox2 inhibitors primarily suppress inflammatory prostaglandins and leave physiological prostaglandins relatively unaffected [36]. It is assumed that inflammatory prostaglandins are powerful regulators, along with BMPs, of the formation of ossificates [2]. Inhibitors of Cox2 and NSAIDs, in addition to their effect on inflammatory prostaglandins, can suppress the migration and proliferation of inducible MSCs [37]. In an animal model of HO induced by BMP-demineralized bone matrix, Cox2 inhibitors and NSAIDs effectively attenuated ectopic bone formation by inhibiting the synthesis of inflammatory prostaglandins [38].

However, Cox2 inhibitors and NSAIDs have shown minimal effect on the arrest or delay of the ectopic bone growth. Most experts agree that indomethacin is the best choice among NSAIDs not only to prevent HO, but also to slow down its development [39]. However, the use of NSAIDs has been limited due to side effects such as peptic ulcer disease, decreased platelet aggregation, and renal failure. Long-term use of NSAIDs increases the risk of developing cardiovascular diseases, and also slows down fracture healing [40]. Previously, prevention of HO with the use of Cox2 inhibitors and NSAIDs was widely used after total hip arthroplasty and arthroscopy, traumatic brain injury, spinal cord injury, etc. [36].

Bisphosphonates inhibit bone remodeling and exert their primary effect by shortening the lifespan of osteoclasts. Bisphosphonates are used to treat numerous bone disorders in which bone loss (resorption) exceeds bone formation, such as osteoporosis, osteogenesis imperfecta, and oncology. The effectiveness of sodium etidronate in the treatment of HO after spinal cord injury has been proven. Sodium etidronate inhibits osteoid mineralization, binds to calcium phosphate, and prevents hydroxyapatite crystallization [41].

Radiation therapy (X-ray therapy) is another effective method for the prevention of HO. It is believed that radiation therapy prevents the formation and/or progression of HO by inhibiting the differentiation of MSCs or osteoprogenitor cells [42]. In particular, in vitro studies demonstrated that radiation therapy suppresses BMP-2 signaling, reduces the proliferation and differentiation of osteoblasts, and promotes their apoptosis [43]. In a preclinical study, adult rats with implanted demineralized bone matrix were treated by radiation 2, 4, 6, 8, 10, and 12 days after implantation [44]. It was found that ectopic bone formed in rats 11 days after implantation. However, it was noted that rats exposed to radiation 2 and 4 days after implantation had decreased HO volume, by ~ 60 and 24 %, respectively. However, when radiation was delayed until 8 days after implantation, the authors did not observe any difference in HO volume between treated by radiation rats and controls. Several studies reported positive effects of radiotherapy on the HO prevention after brain and spinal cord injuries. Thus, in one of the phase I/II clinical trials, 33 patients with HO after spinal cord injury who underwent radiation therapy did not show further growth of ectopic bone; however, joint mobility was slightly impaired in three patients [45].

However, a possible side effect that clinicians should consider is carcinogenesis. However, to date,

there are no documented cases of radiation-induced tumors after radiation therapy used for HO prevention. It is believed that tumors do not occur due to the low dosage of radiation. Another serious complication of radiation therapy is slow bone tissue regeneration, and namely, nonunion of fractures. According to a study by Morcos et al. impaired bone fracture repair may occur in 12–30 % of cases after radiotherapy [46].

Currently, surgical resection is the only effective treatment for HO. However, it is recommended that surgery be considered only if patients with HO meet the following criteria: (1) significant reduction in the range of motion due to joint ankylosis; (2) no acute inflammatory response; (3) mature heterotopic ossificate (sufficiently mineralized) to be removed [47, 48]. However, there are several other factors to consider in choosing the timing of surgery. Previous studies showed that in order to reduce the risk of recurrence, surgical removal is preferable after complete mineralization of the ectopic bone [49]. However, over the last decade there has been a shift in the management of patients with HO in the direction of earlier resection of ectopic bone, i.e. surgery is performed as soon as the patient is stable enough and the lesion is mineralized enough to allow resection [50]. However, recent results reported in clinical studies suggest that early excision of ectopic bone may reduce the risk of surgical complications (e.g., perioperative fracture) or negative neuromuscular changes (e.g., muscle atrophy) [50]. However, resection of ectopic bone is associated with a number of complications. Thus, complete removal of periarticular ossification is particularly problematic because patients may have a persistent reduction in the range of motion. Like any other invasive procedure, ectopic bone resection is associated with potential blood loss during surgery and possible infectious complications in the postoperative period. Moreover, surgical removal may damage adjacent peripheral tissues [51]. Thus, surgery is not the optimal therapy for HO and should be carefully considered.

5. New potential methods of HO prevention and treatment

Currently, the development of the most effective drugs for the prevention and treatment of HO is underway to study the expression of BMP-Smad signaling molecules [2]. Drugs that address this problem include, among others, retinoic acid receptor (RAR) agonists, BMPs antagonists, and substances that affect Smad phosphorylation. Besides, the preventive effect of antioxidants and acceptors of free radicals is being studied.

RAR has three isoforms (RARα, RARβ and RARγ) and is a potent inhibitor of chondrogenesis. Stimulation of this receptor is achieved through the use of its agonists: a non-selective active form of vitamin A and a selective RARγ agonist that block chondrogenesis in vitro [52]. However, inhibition of RARα and RARβ does not affect the process of chondrogenesis. The RAR-γ agonist palovarotene has been shown to be effective in preventing the initial stages of HO *in vitro* and *in vivo* [53]. Palovarotene significantly slows down the HO process by inhibiting the growth and differentiation of osteoprogenitor cells into chondrocytes [53]. Moreover, palovarotin therapy was found to suppress the expression of SRY-box transcription factor 9 (Sox-9) and collagen 2α1 in chondrocytes and the expression of osteocalcin, osteopoietin, BMP-2, BMP-4, POU class 5 homebox 1 (POU5FL) and Runt-associated transcription factor 2 (RUNX2) in osteoblasts. It has also been shown that the administration of RARγ agonists leads to a decrease in BMPs signaling by reducing the phosphorylation of Smad 1, Smad 5, Smad 8 and, possibly, their destruction in the proteasomes. This effect may persist and permanently inhibit the differentiation of MSCs into cells with osteogenic potential [54, 55].

Another strategy targeting the BMP-Smad pathway that may be useful for the prevention of HO is to reduce the level of phosphorus available in the form of adenosine triphosphate (ATP) or adenosine diphosphate (ADP). As stated above, phosphorylation of Smad 1, Smad 5, and Smad 8 leads to the inhibition of differentiation of MSC into the cells with osteogenic potential [55]. Results of *in vitro* and *in vivo* studies of HO models in which HO resulted from a burn injury show that topical application of apyrase, hydrolyzing ATP and ADP reduces the likelihood of ectopic bone formation. Moreover, no osteopenia was observed in the apyrase group [56]. In addition, BMPs receptor antagonists are a group of substances that have the potential to inhibit the HO formation. Thus, LDN-193189 (a potent inhibitor of BMPs signaling pathways that inhibits ALK 1, 2, 3, and 6) inhibits BMP-1 expression and effectively reduces the differentiation of MSCs into osteoblasts in a mouse model of HO [57].

Another BMPS antagonist, Noggin (NOG), may also be considered as a prophylaxis for HO. One of the studies demonstrated the delivery of NOG into the cell using a viral vector, and NOG effectively suppressed the expression of BMP-4 and, thus, the HO procession [58]. However, due to potential side effects, the developed BMPs inhibitors do not have a sufficient safe action profile for the use in human clinical trials.

Recent studies have associated ectopic bone formation not only with inflammation, but also with angiogenic factors released in tissue hypoxia. Pharmacological inhibition of hypoxia-inducible factor 1-alpha (HIF-1α) in the cells of people with advanced fibrodysplasia ossificans has been shown to decrease BMPs signaling. Also in an *in vivo* HO model with an active type I activin A receptor (ACVR1),

pharmacological inhibition of HIF-1α inhibits the HO process. Pharmacological inhibition of HIF-1α using PX-478 or rapamycin also significantly slowed down the HO progression [59, 60].

CONCLUSION

BMPs have a promising potential in the repair of bone defects after injury due to their superior osteoinductive ability. However, like other growth factors, their delivery must be optimized in terms of administered dose and location in the defect zone in order to increase efficiency and reduce side effects associated with the pleiotropic effect of BMPs when they are present in the systemic circulation. Of particular concern are the results of recent clinical studies demonstrating ectopic bone formation associated with the use of rhBMP-2 and rhBMP-7 in orthopedic surgery. According to the rhBMP-2 and rhBMP-7 commercial product safety database, the rate of adverse effects in regard to HO ranges from 1 to 10 %. However, to date, the clinical use of rhBMP-2 and rhBMP-7 has been justified, especially if alternative bone graft substitutes are not available. Despite the numerous studies that have been conducted over the past decades, further scientific investigation of BMPs will allow us to better evaluate long-term outcomes, identify new alternative carriers, and explore the cost-effectiveness of rhBMPs in clinical practice. Finally, we would like to emphasize that a better understanding of the HO process will help clinicians in its prevention and treatment, including after the use of rhBMPs.

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