# Does exogenously adding heparanase accelerate bone healing?

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In the March, August, and September issues of the *Journal of the Chinese Medical Association*, the term of "heparanase" seems to attract our eye to revisit this molecule.[1–3] As shown by editorial comments by Wang and Chen[3] addressing the osteogenesis process, the role of heparanase 1 seemed to be opposite, because some studies favored the enhanced osteogenesis, but some studies found the inhibition of heparanase during osteogenesis. The study from Chiu and colleagues[1] tried to evaluate the possible cause to induce the aforementioned conflicted data, and the authors found that low-dose heparanase can accelerate the bone remodeling, but high dose of heparanase may block the remodeling process. Although editors (Drs. Wang and Chen[3]) have commented that the optimal dosage, route, and application period are very important, because all are critical to avoid overdose-related adverse events, they highlighted that the dosage of heparanase might be the paramount and important key factor on osteogenesis.[3] Does any evidence support the above-mentioned hypothesis? In fact, it is still undetermined what is "appropriate dosage" or "appropriate amount" of heparanase good for the osteogenesis process. In addition, when the above-mentioned dosage of heparanase should be appeared in the "appropriate" period? To study the dynamic, continuous, and overlapped processes, healing or repair as the best example, characterized by inflammation, proliferation, and remodeling triple phases,[4,5] should face the biggest challenge, because it is hard to use "one point" to explain the entire process. Therefore, the current study is worthy of further evaluation and discussion.

Heparanase, produced as an inactive precursor proheparanase (65 kDa), is the sole endo-β-glucuronidase that cleaves heparin sulfate (HS) polysaccharide chains (glycosaminoglycan) in heparin sulfate proteoglycans (HSPGs) into small fragments.[6] Therefore, heparanase regulates the function of HS and is implicates in the crosstalk between cells and their microenvironment.[6] As a subsequence, heparanase contributes to tissue morphogenesis, differentiation, and homeostasis.[6] Although heparanase has the well-known straightforward function to loosen the extracellular matrix (ECM) and breakdown the base membrane (BM), the role of heparanase is much more complicated, either mediated by enzymatically active form or via inactive form.[6] Heparanase not only promotes signal transduction, such as protein kinase B (Akt), signal transducer and activator of transcription (STAT), steroid receptor coactivator (Src), extracellular signal-regulated kinase (Erk), insulin-like growth factor, and epidermal growth factor receptor (EGFR) signaling, but also regulates the transcription of proangiogenic, such as vascular endothelial growth factor (VEGF)-A or C, cyclooxygenase-2 (COX-2), matrix metalloproteinases 9 (MMP-9); prothrombotic (ie, tissue factor); proinflammatory, including tumor necrosis factor α (TNF-α), interleukin 1 (IL-1), and interleukin 6 (IL-6); profibrotic, such as tumor growth factor β (TGF-β); mitogenic (ie, hepatocyte growth factor [HGF]); osteolytic (receptor activator of nuclear factor κ-B ligand [RANKL]); osteoblastic, including basic fibroblast growth factor-2 (bFGF-2), bone morphogenetic proteins (BMPs), and Wnt; and other genes, and all suggest that heparanase has extended much beyond tissue invasion and expanded its functional repertoire and mode of action, and furthermore, heparanase has shown the significant but dynamical impacts on multiple cellular processes and biological activities.[6,7] Heparanase may have a dual function. Therefore, there is no doubt that the diverse results from the different studies for the evaluation of the bone healing process can be found.[1,3]

Second, it is also well known that heparanase is critically required for activation and function of macrophages[6] that play an essential constituent of the microenvironment that may enhance or interrupt the repairing or healing process after attacks of trauma or injury. Besides macrophages, other immune cells, endothelial cells, and hematological cells, such as platelets in the microenvironment, are also modulated either by enzymatic or nonenzymatic activity of heparanase, suggesting that heparanase is also involved in inflammatory/hemostasis at the initial phase of healing process. Without the presence of more much solid data, it is still challenged when the low dose of heparanase could be applied to accelerate the bone healing process.

Third, in an in vitro study, an exogenous use of heparanase can induce osteogenic differentiation of cultured MC3T3 E1 osteoblastic cells, and in addition, a long-term heparanase treatment to disrupt cell surface HSPGs expression can increase osteogenic differentiation of the human mesenchymal stem

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cells via increasing BMP and Wnt activity.[8] Furthermore, in a transgenic mice overexpressing human heparanase model, these transgenic mice displayed the increased trabecular bone mass and increased bone formation rate.[9,10] All suggest that heparanase might play a positive role during the osteogenesis process. However, much evidence supports that the role of heparanase might work as a "bad guy," because its overexpression often occurs in multiple pathological processes, such as aggressive behavior and worse prognosis in cancer, and purulent inflammation, kidney dysfunction, and atherosclerosis formation.[6,7,11] Therefore, the role of heparanase in the bone healing process is still controversial. For example, studies have shown that heparanase significantly inhibited osteoblast differentiation and mineralization and reduced bone formation in vivo, and additionally, shifted the differentiation potential of osteoblast progenitors from osteoblastogenesis to adipogenesis.[12] It is interesting to find that the heparanase knockout (KO) mice were fertile, exhibited a normal life span, and did not have apparent pathological changes, although the significant alterations in the expression level of MMP family members, primarily a marked elevation of MMP-2 and MMP-14, have been found,[13,14] suggesting that the use of heparanase as a target for drug development is promising, similar to many enzymes playing the modulation role in both normal and pathological processes,[15] because KO expression of heparanase will be viable, and of the most importance, KO expression of heparanase is absent of significant abnormalities.[14]

Fourth, another form of heparanase should be considered in the discussion about the role of heparanase. For example, heparanase 2 has been cloned and identified in 2000, which consisted of 2353 base pairs encoding a protein of 592 amino acids (aa), and could be secreted, likely due to extra glycosylation sites.[6,16] Compared with conventional heparanase (often called as heparanase 1), heparanase 2 is total loss of intrinsic HS-degrading activity, contributing to absent proteolytic processing ability.[6,16] In addition, several variants due to different splicing of the heparanase 2 protein transcript have also been identified, including heparanase 2a (480 aa), and heparanase 2b (534 aa), and all of which were totally absent of extraglycosylation sites.[14] Only heparanase 2 (also called as heparanase 2c) retains the capacity to bind heparin/HS and, of most importance, exhibits a much higher affinity toward heparin/HS than heparanase. Thus, the competing HS binding and inhibiting heparanase enzyme activity hint that the heparanase 2 family may function as a tumor suppressor.[6]

Taken together, we found that the significance of heparanase in health and disease may be underestimated, because of its dramatic various and complex modes of action. With continuous advances in basic and translational study for the heparanase, heparanase not only works on the breakdown of ECM and BM but also is involved in the regulation and bioavailability of growth factors, cytokines and many signal transduction pathways. We are looking forward to seeing more studies exploring this field.

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## REFERENCES

1. Chiu PY, HuangFu WC, Liu IH, Chang YP. Topical application of Heparanase-1 facilitates bone remodeling during the healing of bone defects in a mouse model. *J Chin Med Assoc* 2020;**83**:272–9.
2. Lin LT, Lee WL, Wang PH. Healing. *J Chin Med Assoc* 2020;**83**:695–6.
3. Wang JY, Chen CF. Heparanase-1 facilitates bone remodeling on bone defect animal model with optimal dosage and treatment duration. *J Chin Med Assoc* 2020;**83**:799–800.
4. Huang CY, Chang WH, Cheng M, Huang HY, Horng HC, Chen YJ, et al. Crosslinked hyaluronic acid gels for the prevention of intrauterine adhesions after a hysteroscopic myomectomy in women with submucosal myomas: a prospective, randomized, controlled trial. *Life (Basel)* 2020;10:67.
5. Horng HC, Chang WH, Yen CC, Huang BS, Chang CP, Chen YJ, et al. Estrogen effects on wound healing. *Int J Mol Sci* 2017;**18**:2325.
6. Vlodavsky I, Gross-Cohen M, Weissmann M, Ilan N, Sanderson RD. Opposing functions of heparanase-1 and heparanase-2 in cancer progression. *Trends Biochem Sci* 2018;**43**:18–31.
7. Aldi S, Eriksson L, Kronqvist M, Lengquist M, Löfling M, Folkersen L, et al. Dual roles of heparanase in human carotid plaque calcification. *Atherosclerosis* 2019;**283**:127–36.
8. Manton KJ, Leong DF, Cool SM, Nurcombe V. Disruption of heparan and chondroitin sulfate signaling enhances mesenchymal stem cell-derived osteogenic differentiation via bone morphogenetic protein signaling pathways. *Stem Cells* 2007;**25**:2845–54.
9. Zcharia E, Metzger S, Chajek-Shaul T, Aingorn H, Elkin M, Friedmann Y, et al. Transgenic expression of mammalian heparanase uncovers physiological functions of heparan sulfate in tissue morphogenesis, vascularization, and feeding behavior. *FASEB J* 2004;**18**:252–63.
10. Kram V, Zcharia E, Yacoby-Zeevi O, Metzger S, Chajek-Shaul T, Gabet Y, et al. Heparanase is expressed in osteoblastic cells and stimulates bone formation and bone mass. *J Cell Physiol* 2006;**207**:784–92.
11. Tatsumi Y, Miyake M, Shimada K, Fujii T, Hori S, Morizawa Y, et al. Inhibition of heparanase expression results in suppression of invasion, migration and adhesion abilities of bladder cancer cells. *Int J Mol Sci* 2020;**21**:3789.
12. Ruan J, Trotter TN, Nan L, Luo R, Javed A, Sanderson RD, et al. Heparanase inhibits osteoblastogenesis and shifts bone marrow progenitor cell fate in myeloma bone disease. *Bone* 2013;**57**:10–7.
13. Vlodavsky I, Ilan N, Sanderson RD. Forty years of basic and translational heparanase research. *Adv Exp Med Biol* 2020;**1221**:3–59.
14. Zcharia E, Jia J, Zhang X, Baraz L, Lindahl U, Peretz T, et al. Newly generated heparanase knock-out mice unravel co-regulation of heparanase and matrix metalloproteinases. *PLoS One* 2009;**4**:e5181.
15. Sung PL, Wen KC, Horng HC, Chang CM, Chen YJ, Lee WL, et al. The role of α2,3-linked sialylation on clear cell type epithelial ovarian cancer. *Taiwan J Obstet Gynecol* 2018;**57**:255–63.
16. McKenzie E, Tyson K, Stamps A, Smith P, Turner P, Barry R, et al. Cloning and expression profiling of Hpa2, a novel mammalian heparanase family member. *Biochem Biophys Res Commun* 2000;**276**:1170–7.