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Histological analysis of Isradipine, a calcium channel inhibitor, on wound healing following dental extraction in rats

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ABSTRACT. The use of drugs to promote bone regeneration and accelerate tissue repair is a growing field of research, especially in cases of significant bone loss. Isradipine, a calcium channel blocker traditionally used to treat hypertension, has shown potential effects on bone metabolism, suggesting a possible role in promoting bone repair processes. The present study investigated bone repair after tooth extraction in Wistar rats and assessed the effects of Isradipine administration. Forty-eight male rats, approximately 90 days old and weighing an average of 250 g, were randomly divided into two groups: Control and Experimental. In the Control group, only the extraction of the upper left central incisor was performed, whereas in the Experimental group, Isradipine (2.5 mg) was administered intraperitoneally after tooth extraction. The animals were euthanized at days 3, 7, 14, and 21 for histological analysis of bone repair. Histological analysis revealed that, at 3 days, both groups showed sockets filled with blood clots and initial inflammatory cells, without bone trabeculae. By day 7, the Control group exhibited immature osteoid tissue, whereas the Experimental group had a greater number of fibroblasts. At days 14 and 21, bone formation advanced in both groups, with denser bone tissue observed in the Experimental group. These findings underscore the potential of Isradipine to enhance bone repair and suggest promising avenues for the application of calcium channel blockers in bone tissue bioengineering.

Keywords: Bone regeneration; Calcium channels; Bone metabolism.

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Introduction

The use of drugs that promote bone regeneration and the formation of new tissue has been widely investigated (Xing et al., 2023), often with the aim of accelerating repair processes in areas with significant bone loss (Song et al., 2024). The prescription of medications in the context of bone repair has gained prominence due to their ability to enhance the patient's biological response (Drake et al., 2019), optimizing the healing process (Rothe et al., 2020; Vinikoor et al., 2023), improving graft integration, and reducing recovery time (Lebaudy et al., 2021; Chen et al., 2024).

Although many of these drugs can influence bone metabolism, a significant portion was originally intended for the treatment of other clinical conditions, which has led to the exploration of a wide range of medications for their potential benefits in bone healing (Kehoe et al., 2019). Isradipine, a drug traditionally used in the treatment of hypertension (Hansson, 1990; Wang et al., 2017; Mohapatra et al., 2022), is a calcium channel blocker (Alam et al., 2022) that has shown significant effects on bone metabolism. It can act on osteoclasts, increasing intracellular calcium concentration, which results in reduced bone resorption (Ritchie et al., 1994; Okada et al., 2020).

Since the 1980s, interest in this drug's action has grown, with studies focusing on its effects on plasma levels of aldosterone and other hormones (Abernethy, 1988; Stölting et al., 2023), as well as its potential anti-atherogenic effects (Heider et al., 1987; Weinstein & Heider, 1987; Orekhov et al., 1988; Hof et al., 1989), and its ability to inhibit platelet aggregation. These studies have highlighted the importance of Isradipine not only in blood pressure control but also in aspects related to the modulation of local vascular responses and cellular proliferation.

In the present study, we investigated the potential effects of Isradipine on the wound healing timeline following dental extraction, assessing alveolar tissue repair in rats subjected to tooth extraction and treated with this calcium channel blocker (Lomir®).

Materials and methods

Forty-eight male Wistar rats (*Rattus norvegicusalbinus*, Wistar), approximately 90 days old and weighing around 250 g, were used in this study. After weighing, the animals were sedated using inhaled sulfuric ether vapor and randomly divided into two groups (n = 24): a) Control group (C): Animals that underwent extraction of the upper left central incisor (Okamoto, 1973); b) Experimental group (I): After the tooth extraction procedure, the drug Isradipine (Lomir 2.5 mg Ml^{-1}, Sandoz Pharma S.A., Basel, Switzerland) was administered intraperitoneally in a single dose, calculated based on the animals' body weight. Immediately after extraction, the sockets in both groups were sutured using no. 4 silk suture thread (Sutupack-Ethicon, Johnson & Johnson).

Euthanasia was performed on days 3, 7, 14, and 21 after tooth extraction. Subsequently, the left maxilla was separated from the right by an incision along the median sagittal plane, following the intermaxillary suture. A tangential cut with straight scissors along the distal face of the molars enabled removal of the sample containing the left tooth socket. The samples were fixed in 10% formalin for 24 hours and decalcified over approximately 30 days in a solution of sodium citrate and formic acid in equal parts. After decalcification, the samples were dehydrated, cleared, and embedded in paraffin, with orientation to allow sectioning of the socket in the vestibulolingual direction, parallel to its long axis (Figure 1).

Semi-serial sections, 6 µm thick, were obtained from each block and stained with hematoxylin and eosin. Photomicrographs were captured using a Zeiss Axioskop 40 optical microscope (Carl Zeiss). Five images per sample were captured and analyzed by two calibrated, blinded examiners using a light microscope for visualization. The evaluated parameters included inflammation, new bone formation, and the pattern of bone trabeculae, comparing results across the analyzed time points.

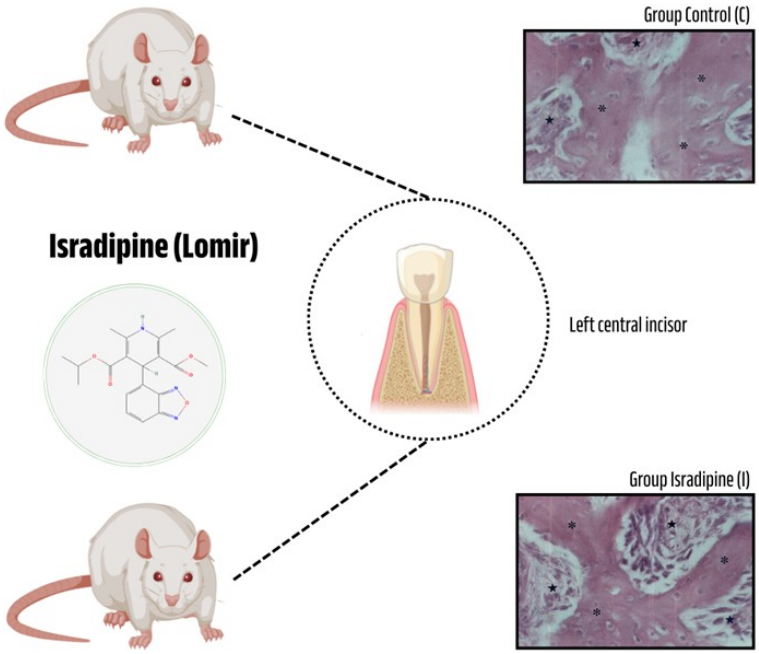

Figure 1. Schematic diagram of procedure.

Results

The histological characteristics of the evaluated groups across all time points are shown in Figure 2. At day 3, Group C presented an alveolar socket almost completely filled with blood clot. Along the socket walls, near the periodontal ligament, young fibroblasts and remnants of the periodontal ligament were observed. Cells characteristic of an early inflammatory process, such as neutrophils and leukocytes, were also noted. In Group I, histological features were similar to those observed in Group C. Intense fibroblastic proliferation was evident along the socket walls near the remaining periodontal ligament, with the socket still predominantly filled with blood clot. During this initial period, bone trabeculae or cells involved in bone repair were not yet observed. At day 7, Group C displayed a large number of fibroblasts, which give rise to amorphous ground substance (Figure 2 - Black arrow). There were also areas with blood clots and the formation of immature and disorganized osteoid tissue, as well as the presence of newly formed capillaries along the socket. In contrast,

Group I showed a significant amount of blood clot and an abundance of mature fibroblasts, with extensive presence of amorphous ground substance and a small amount of osteoid tissue.

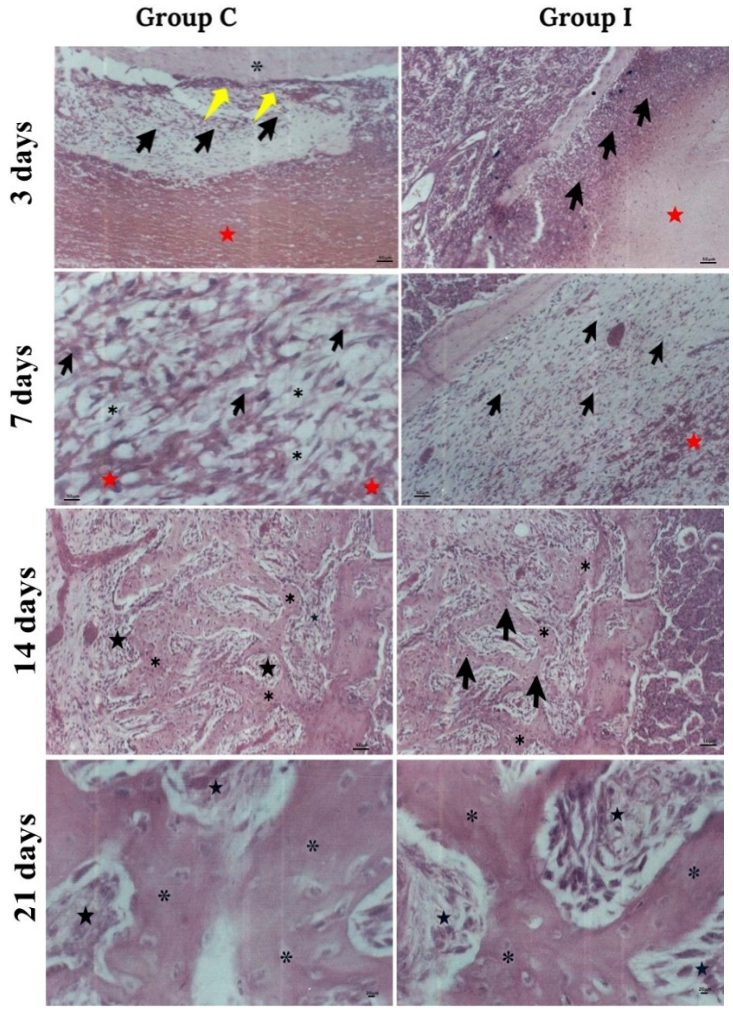

Figure 2. Histological section. Legend: Red star: clot; Yellow arrow: remnants of the periodontal ligament adjacent to the alveolar bone; Black arrow: presence of fibroblasts; Black star: connective tissue; Asterisk: bone tissue. Hematoxylin and eosin staining; original magnification: ×63 (scale bar = 50 µm) and x250 (scale bar = 20 µm).

With the extension of the analysis period to 14 days, a marked change was observed in the histological profile of the socket, showing intense bone activity in Group C, where the socket was almost completely filled with bone tissue at different stages of mineralization, and numerous osteoblasts present. The same histological pattern was observed in Group I, with a decrease in the presence of fibroblasts. In both groups, disorganized clots and more mature connective tissue were present between areas of newly formed bone tissue.

At day 21, Group C presented a socket filled with dense and organized bone tissue, showing areas of rounded bone tissue in the form of spicules surrounded by connective tissue and blood capillaries, which characterizes the mineralization phase of bone formation. Group I exhibited even denser and more organized bone tissue than Group C. Additionally, larger amounts of newly formed capillaries were observed along the socket, as well as remnants of the clot in the spaces between the newly formed trabeculae.

Discussion

In this study, a histological evaluation was conducted to investigate the influence of Isradipine (Lomir®), a calcium channel blocker, on the timeline and quality of alveolar repair in extraction wounds in rats. The aim was to elucidate the histological changes induced by Isradipine administration following dental extraction, providing a deeper understanding of the drug's effects on the alveolar healing process.

A pioneering study by Ritchie et al. (1994), published in the *Journal of the Endocrine Society*, demonstrated that calcium channel blockers can induce changes in bone resorption through their action on osteoclasts. The

authors suggested that these drugs directly interact with calcium channels in osteoclasts, resulting in an increase in intracellular calcium concentration and, consequently, a reduction in bone resorption. However, the exact mechanism remains unclear, and the literature on the effects of antihypertensive drugs on bone function—especially in animal models—is still limited. In this context, four key phases in the timeline of alveolar bone healing have been identified as critical for the progression of repair: cell proliferation, development and maturation of connective tissue, and bone differentiation or mineralization (Araújo et al., 2015; Mardas et al., 2023). These phases do not occur in isolation; by the seventh day after extraction in rats, all four phases can be observed histologically, as noted in both groups. These findings contribute to the understanding of Isradipine's effects on alveolar bone repair, opening new perspectives for future research in biomedical and tissue engineering.

A recent study by Nagasamy Venkatesh et al. (2021) evaluated immediate-release and sustained-release Isradipine tablets, finding that the sustained-release formulation showed more promising results in terms of absorption compared to the immediate-release version. In the present study, the similar histological patterns observed in both groups may be potentially related to the pharmacokinetics of intraperitoneally administered Isradipine.

It is important to highlight that combining synthetic biomaterials with drugs has gained significant attention in recent years, offering new perspectives in tissue regeneration (Dimatteo et al., 2018; Song et al., 2024). Ma et al. (2015) emphasized the beneficial use of benidipine (BD), another calcium channel blocker, which promoted mesenchymal cell differentiation into osteoblasts. They also demonstrated positive effects of this drug in an ovariectomized mouse model (OVX), showing significant differences in microcomputed tomography evaluations ($p<0.01$) between the OVX+BD and the OVX groups.

In this study, a rat dental extraction model was used, and no significant differences in histological findings were observed, as both groups exhibited similar healing. The pioneering research of Euler (1923)initiated investigations into the repair process following dental extraction and, since then, various researchers have explored this biological phenomenon through experimental and clinical studies (Imai et al., 2019). These studies employed radiographic, histological, and histochemical methods, which were instrumental in establishing healing and regeneration patterns following dental extractions. Another crucial factor to consider is the potential influence of these drugs on local vascular proliferation (Cunha, 2000; França et al., 2014; Kim et al., 2023), a critical process for optimal healing. Calcium channel blockers, particularly those from the dihydropyridine class, relax arterial and arteriolar smooth muscles, potentially improving blood supply to the alveolar region and positively influencing the repair process. A greater quantity of microvascular vessels present in the bone marrow has been associated with increased bone mass in mice (Xiao et al., 2024). Calcium channel blockers can significantly impact bone metabolism and inflammation, highlighting the crucial role of calcium and its modulators in bone health (Barradas et al., 2012; Ma et al., 2015).

Studies on Isradipine have been highlighted due to innovations in the formulation of a drug that avoids the side effects associated with previously used antihypertensive treatments. This study contributes to expanding knowledge about the use of this drug in other fields, as current understanding of its impact on bone metabolism remains limited, requiring the development of new evaluation approaches and methodologies. Despite the inherent limitations of this study, the results provide a valuable starting point for investigating the application of calcium channel blockers in bone tissue bioengineering. To advance this field, it is essential to integrate new methodologies, explore innovative approaches, and investigate the association of this drug with other biomaterials to improve understanding and therapeutic applications.

Conclusion

As an initial investigation, the results of using Isradipine in bone repair appear promising. Despite its limitations, this study opens a line of research into the potential of calcium channel blockers in bone metabolism. Future studies may explore the application of Isradipine in contexts of systemic impairment of tissue repair, such as osteoporosis, to broaden understanding of its therapeutic role in bone disorders

References

Abernethy, D. R., Gutkowska, J., & Lambert, M. D. (1988). Amlodipine in elderly hypertensive patients: Pharmacokinetics and pharmacodynamics. *Journal of Cardiovascular Pharmacology*, *12*, 67–71.

Alam, T., Ansari, M. A., Baboota, S., & Ali, J. (2022). Nanostructured lipid carriers of isradipine for effective management of hypertension and isoproterenol induced myocardial infarction. *Drug Delivery and Translational Research*, *12*(3), 577–588. https://doi.org/10.1007/s13346-021-00958-x

Araújo, M. G., Silva, C. O., Misawa, M., & Sukekava, F. (2015). Alveolar socket healing: What can we learn? *Periodontology 2000*, *68*(1), 122–134. https://doi.org/10.1111/prd.12082

Barradas, A. M. C., Fernandes, H. A. M., Groen, N., Chai, Y. C., Schrooten, J., van de Peppel, J., van Leeuwen, J. P. T. M., van Blitterswijk, C. A., & de Boer, J. (2012). A calcium-induced signaling cascade leading to osteogenic differentiation of human bone marrow-derived mesenchymal stromal cells. *Biomaterials*, *33*(11), 3205–3215. https://doi.org/10.1016/j.biomaterials.2012.01.020

Chen, L., Jiang, C., Xu, Q., Jin, J., A, S., Wang, X., Li, X., Hu, Y., Sun, H., Lu, X., Duan, S., Gao, Z., Wang, W., & Wang, Y. (2024). Biphasic release of betamethasone from an injectable HA hydrogel implant for alleviating lumbar disc herniation induced sciatica. *Acta Biomaterialia*, *176*, 173–189. https://doi.org/10.1016/j.actbio.2024.01.016

Cunha, R. F. A. V. L. (2000). Renin-angiotensin-aldosterone system and hypertensive vascular lesion. *Revista Brasileira de Hipertensão*, *3*, 282–292.

Dimatteo, R., Darling, N. J., & Segura, T. (2018). In situ forming injectable hydrogels for drug delivery and wound repair. *Advanced Drug Delivery Reviews*, *127*, 167–184. https://doi.org/10.1016/j.addr.2018.03.007

Drake, M. T., Cremers, S., Russell, R. G., & Bilezikian, J. P. (2019). Drugs for the treatment of metabolic bone diseases. *British Journal of Clinical Pharmacology*, *85*(6), 1049–1051. https://doi.org/10.1111/bcp.13857

Euler, H. (1923). Die Heilung von Extraktionswunden. *Deutsche Monatsschrift für Zahnheilkunde*, *41*, 685–689.

França, C. N., Izar, M. C. O., Amaral, J. B., Tegani, D. M., & Fonseca, F. A. H. (2014). Microparticles as potential biomarkers of cardiovascular disease. *Arquivos Brasileiros de Cardiologia*. https://doi.org/10.5935/abc.20140210

Hansson, L. (1990). Isradipine in hypertension. *Drugs*, *40*(Suppl. 2), 10–14. https://doi.org/10.2165/00003495-199000402-00005

Heider, J. G., Weinstein, D. B., Pickens, C. E., Lan, S., & Su, C. M. (1987). Anti-atherogenic activity of the calcium channel blocker isradipine (PN-200-110): A novel effect on matrix synthesis independent of calcium channel blockade. *Transplantation Proceedings*, *19*(4 Suppl. 5), 96–101.

Hof, H. P., Salzmann, R., & Siegl, H. (1989). Selective effects of PN 200-110 (isradipine) on the peripheral circulation and the heart. *American Journal of Cardiology*, *59*, 30B–6B.

Imai, M., Ayukawa, Y., Yasunami, N., Furuhashi, A., Takemura, Y., Adachi, N., Hu, J., Zhou, X., Moriyama, Y., Atsuta, I., Kurata, K., & Koyano, K. (2019). Effect of a single injection of benidipine-impregnated biodegradable microcarriers on bone and gingival healing at the tooth extraction socket. *Advances in Wound Care*, *8*(3), 108–117. https://doi.org/10.1089/wound.2018.0834

Kehoe, T., Blind, E., & Janssen, H. (2019). Regulatory aspects of the development of drugs for metabolic bone diseases – FDA and EMA perspective. *British Journal of Clinical Pharmacology*, *85*(6), 1208–1212. https://doi.org/10.1111/bcp.13791

Kim, Y., Clemens, E. G., Farner, J. M., Londono-Barbaran, A., Grab, D. J., & Dumler, J. S. (2023). Spotted fever rickettsia-induced microvascular endothelial barrier dysfunction is delayed by the calcium channel blocker benidipine. *Biochemical and Biophysical Research Communications*, *663*, 96–103. https://doi.org/10.1016/j.bbrc.2023.04.045

Lebaudy, E., Fournel, S., Lavalle, P., Vrana, N. E., & Gribova, V. (2021). Recent advances in anti-inflammatory material design. *Advanced Healthcare Materials*, *10*(1). https://doi.org/10.1002/adhm.202001373

Ma, Z., Liao, J., Zhao, C., & Cai, D. (2015). Effects of the 1,4-dihydropyridine L-type calcium channel blocker benidipine on bone marrow stromal cells. *Cell and Tissue Research*, *361*(2), 467–476. https://doi.org/10.1007/s00441-015-2115-x

Mardas, N., Macbeth, N., Donos, N., Jung, R. E., & Zuercher, A. N. (2023). Is alveolar ridge preservation an overtreatment? *Periodontology 2000*, *93*(1), 289–308. https://doi.org/10.1111/prd.12508

Mohapatra, P. K., Srivastava, R., Varshney, K. K., & Babu, S. H. (2022). Formulation and evaluation of Isradipine nanosuspension and exploring its role as a potential anticancer drug by computational

approach. *Anti-Cancer Agents in Medicinal Chemistry*, *22*(10), 1984–2001. https://doi.org/10.2174/1871520621666210805125426

Nagasamy Venkatesh, D., Meyyanathan, S. N., Shanmugam, R., Kamatham, S. S., Campos, J. R., Dias-Ferreira, J., Sanchez-Lopez, E., Cardoso, J. C., Severino, P., & Souto, E. B. (2021). Physicochemical, pharmacokinetic, and pharmacodynamic characterization of isradipine tablets for controlled release. *Pharmaceutical Development and Technology*, *26*(1), 92–100. https://doi.org/10.1080/10837450.2020.1839495

Okada, H., Okabe, K., & Tanaka, S. (2020). Finely-tuned calcium oscillations in osteoclast differentiation and bone resorption. *International Journal of Molecular Sciences*, *22*(1), 180. https://doi.org/10.3390/ijms22010180

Okamoto, T. R. M. C. (1973). Wound healing following tooth extraction: Histochemical study in rats. *Revista da Faculdade de Odontologia de Araçatuba*, *2*, 153–169.

Orekhov, A. N., Baldenkov, G. N., Tertov, V. V., Ryong, L. H., Kozlov, S. G., Lyakishev, A. A., Tkachuk, V. A., Ruda, M. Y., & Smirnov, V. N. (1988). Cardiovascular drugs and atherosclerosis: Effects of calcium antagonists, beta-blockers, and nitrates on atherosclerotic characteristics of human aortic cells. *Journal of Cardiovascular Pharmacology*, *12*(Suppl. 6), 66–68.

Ritchie, C. K., Maercklein, P. B., & Fitzpatrick, L. A. (1994). Direct effect of calcium channel antagonists on osteoclast function: Alterations in bone resorption and intracellular calcium concentrations. *Endocrinology*, *135*(3), 996–1003. https://doi.org/10.1210/endo.135.3.8070395

Rothe, R., Hauser, S., Neuber, C., Laube, M., Schulze, S., Rammelt, S., & Pietzsch, J. (2020). Adjuvant drug-assisted bone healing: Advances and challenges in drug delivery approaches. *Pharmaceutics*, *12*(5), 428. https://doi.org/10.3390/pharmaceutics12050428

Song, P., Zhou, D., Wang, F., Li, G., Bai, L., & Su, J. (2024). Programmable biomaterials for bone regeneration. *Materials Today Bio*, *29*, 101296. https://doi.org/10.1016/j.mtbio.2024.101296

Stölting, G., Dinh, H. A., Volkert, M., Hellmig, N., Schewe, J., Hennicke, L., Seidel, E., Oberacher, H., Zhang, J., Lifton, R. P., Urban, I., Long, M., Rivalan, M., Nottoli, T., & Scholl, U. I. (2023). Isradipine therapy in Cacna1dIle772Met/+ mice ameliorates primary aldosteronism and neurologic abnormalities. *JCI Insight*, *8*(20). https://doi.org/10.1172/jci.insight.162468

Vinikoor, T., Dzidotor, G. K., Le, T. T., Liu, Y., Kan, H.-M., Barui, S., Chorsi, M. T., Curry, E. J., Reinhardt, E., Wang, H., Singh, P., Merriman, M. A., D'Orio, E., Park, J., Xiao, S., Chapman, J. H., Lin, F., Truong, C.-S., Prasadh, S., & Nguyen, T. D. (2023). Injectable and biodegradable piezoelectric hydrogel for osteoarthritis treatment. *Nature Communications*, *14*(1), 6257. https://doi.org/10.1038/s41467-023-41594-y

Wang, Q.-M., Xu, Y.-Y., Liu, S., & Ma, Z.-G. (2017). Isradipine attenuates MPTP-induced dopamine neuron degeneration by inhibiting up-regulation of L-type calcium channels and iron accumulation in the substantia nigra of mice. *Oncotarget*, *8*(29), 47284–47295. https://doi.org/10.18632/oncotarget.17618

Weinstein, D. B., & Heider, J. G. (1987). Antiatherogenic properties of calcium antagonists. *The American Journal of Cardiology*, *59*(3), B163–B172. https://doi.org/10.1016/0002-9149(87)90097-X

Xiao, C.-L., Liu, L.-L., Tang, W., Liu, W.-Y., Wu, L.-Y., & Zhao, K. (2024). Reduction of the trans-cortical vessel was associated with bone loss, another underlying mechanism of osteoporosis. *Microvascular Research*, *152*, 104650. https://doi.org/10.1016/j.mvr.2023.104650

Xing, Y., Qiu, L., Liu, D., Dai, S., & Sheu, C.-L. (2023). The role of smart polymeric biomaterials in bone regeneration: A review. *Frontiers in Bioengineering and Biotechnology*, *11*. https://doi.org/10.3389/fbioe.2023.1240861