BioClinix: Journal of Medical and Pharmaceutical Sciences
www.bioclinixjournal.com | VOLUME-1 ISSUE-1 | INAGURAL EDITION | OCTOBER-DECEMEBER 2025 | BI006

PHARMACOLOGICAL EFFECTS OF BENIDIPINE: IN VITRO AND IN VIVO STUDIES

Dr. D. UDAY KUMAR , *Professor,
Department of Pharmacology,
JOGINPALLY B.R. PHARMACY COLLEGE, HYDERABAD.

ABSTRACT: This study consolidates evidence from in vitro and in vivo models to elucidate the pharmacological profile and therapeutic relevance of benidipine. Research involving cells, tissues, and animals has shown the substantial antihypertensive, cardioprotective, nephroprotective, and vasculoprotective properties of benidipine, a long-acting, multi-channel calcium antagonist. Besides its ability to regulate blood pressure, benidipine demonstrates antioxidant, anti-inflammatory, anti-remodeling, and endothelial-stabilizing characteristics, potentially offering broader benefits in cardiac and renal diseases. Comparative analyses demonstrate enhanced tissue selectivity, prolonged efficacy, and reduced adverse effects relative to conventional calcium channel blockers. Due to the variability in research design, dosage selection, and experimental outcomes, meticulously designed clinical trials are necessary to confirm these preclinical results. The report consolidates existing knowledge and delineates prospective strategies to enhance the clinical application of benidipine.

Keywords: *Benidipine; calcium channel blocker; antihypertensive; in vitro; in vivo; pharmacological effects; cardioprotection; nephroprotection; endothelial function; oxidative stress; inflammation; experimental models; review.*

1. INTRODUCTION

Approximately 25% of the global population experiences hypertension, characterized by elevated arterial blood pressure. Significant health issues have arisen; from 1990 to 2015, the number of healthy life years lost surged by 43%, culminating in 212 million lost healthy life years and 9.4 million annual fatalities. For numerous years, individuals have utilized benidipine, an oral long-acting calcium channel blocker derived from dihydropyridine, as an antihypertensive agent. Benidipine effectively inhibits L, N, and T type calcium channels. Benidipine exhibits potent and enduring antihypertensive effects due to its high affinity for the DHP binding site on vascular L-type Ca2+ channels, its capacity to inhibit tachycardia via T-type Ca2+ channels, and its ability to suppress catecholamine release from sympathetic nerves through N-type Ca2+ channels.

It rapidly attained its peak concentration (T max) following oral administration, unlike other calcium channel blockers. In humans, dogs, and rats, benidipine exhibited a binding affinity to plasma proteins exceeding 98%. Basic research, both in vitro and in vivo, has demonstrated Benidipine's antioxidative properties, its ability to stimulate nitric oxide production, inhibit adhesion molecule expression, promote osteoblast differentiation, suppress vascular smooth muscle proliferation, inhibit mesangial cell growth, and provide myocardial protection.

This study investigates the correlation between the pharmacological effects of benidipine both in vivo and in vitro.

2. THERAPEUTIC EFFECTS OF BENIDIPINE — IN VIVO

EFFECT ON BONE

A notable alteration in bone development was detected in tooth extraction sites subsequent to the administration of Benidipine. MRONJ is characterized by an oral bacterial infection that impacts the bone. Benidipine accelerates the recuperation of gingival soft tissue and facilitates bone regrowth. Rats with MRONJ-like regions of tooth extraction sockets received a single administration of benidipine, and alterations in the necrotic extraction socket were assessed using μCT scans and histological analysis. The μCT scan revealed the formation of new bone within the extraction socket. In rats with MRONJ-like symptoms, a singular, targeted injection of BD enhanced the formation of new bone while reducing the surface area of exposed and necrotic bone. Osteoporosis was also noted as a significant clinical issue linked to hypertension and its adverse effects on bone development. The effects of Benidipine on osseous development and bone marrow stromal cells (BMSC) in osteoporotic conditions were examined. In a postmenopausal osteoporosis animal model utilizing ovariectomized (OVX) mice, benidipine treatment increased the expression of β-catenin and LDL receptor-related protein 5 (LRP5). This indicates that the WNT signaling pathway promotes BMSC osteogenesis. Micro-CT studies indicate that Benidipine significantly halted bone loss in OVX animals. Moreover, BD elevated the expression of alkaline phosphatase (ALP), runt-related transcription factor-2 (RUNX2), and osteocalcin (OCN), hence reinforcing its role in promoting bone production and growth. This suggests that benidipine may be an appropriate option for postmenopausal women with hypertension and osteoporosis.

EFFECT ON BRAIN

Cerebral ischemia can result in fatal damage. The intervention for cerebral ischemic injury involves the prompt restoration of blood reperfusion. Nonetheless, reperfusion may lead to brain injury, edema, bleeding, and neuronal death. This phenomenon is termed ischemia/reperfusion. Benidipine therapy significantly reduced cerebral infarction, exhibited antioxidant and anti-apoptotic properties, and diminished lipid peroxidation in rats subjected to ischemia/reperfusion injury. Histological study of the cerebral cortex indicated the neuroprotective characteristics of benidipine. This beneficial effect may be attributed to the decreased overproduction of inflammatory cytokines and the enhancement of antioxidant capacity in cerebral tissue. Seizures frequently occur in individuals with epilepsy, adversely affecting their quality of life. Neuroprotection is crucial for the treatment of generalized tonic-clonic seizures, a prevalent and perilous type of epilepsy, alongside seizure management. Antiepileptic medicines function through many mechanisms, including ion channels (sodium, calcium, and potassium), synaptic vesicles, and GABA regulation. Valproic acid is a drug that influences GABA metabolism and ion channels. Benidipine, a calcium channel blocker, may inhibit neurodegeneration by reducing calcium influx into cells. In an experimental paradigm utilizing pentylenetetrazol, rats received different dosages of benidipine or combinations with valproic acid to investigate the drug's efficacy in treating epilepsy. The Benidipine 4 mg/kg cohort markedly reduced IL-1 levels, however all groups exhibited comparable antiepileptic effects. The cohort exhibiting the lowest TNF-alpha levels was the one administered valproic acid and benidipine at a dosage of 2 mg/kg. Benidipine

CC BY NC

exhibits neuroprotective and antiepileptic characteristics, rendering it a viable and cost-effective treatment for epilepsy.

EFFECT ON HEART

Calcium antagonists used before ischemia reduce infarct size and improve recovery; ischemia followed by reperfusion leads to intracellular calcium overload. Apoptosis is regulated by both internal and external influences. Procaspase-8 is activated via the extrinsic route by ligands including Fas and TNFR1. The intrinsic pathway is initiated by mitochondria, which then release cytochrome c and activate caspases. Benidipine is a vasodilatory calcium antagonist that reduces cytosolic calcium levels, enhances cardiac contractility during ischemia/reperfusion, and decreases myocardial mortality. In rats, benidipine does not influence caspase-8; however, it reduces cytochrome-c release and caspase-9 activation, hence diminishing apoptosis. It enhances and extends ERK1/2 activity, while exerting negligible influence on p38 MAPK. Calcium channel blockers confer cardioprotection in myocardial ischemia/reperfusion by inhibiting post-ischemic myocardial apoptosis. Benidipine primarily mitigates cardiac injury induced by intracellular calcium excess during ischemia and reperfusion by obstructing calcium currents. Intracellular Ca2+ accumulation and lipid peroxidation induced by reactive oxygen species generated during reperfusion result in cellular membrane damage and functional impairments. Benidipine, a dihydropyridine calcium channel blocker, functions as an antioxidant in rat arteries and preserves vascular endothelial cell function during ischemia and reperfusion. In isolated perfused rat hearts, benidipine demonstrated a reduction in myocardial injury during ischemia and reperfusion. This study assessed Benidipine's impact on $H2O_2$-induced myocardial injury in isolated rat hearts, emphasizing the significance of its antioxidant properties within therapeutic dosage parameters. Significantly, benidipine's antagonistic effect on L-type Ca2+ channels enhances coronary blood flow while diminishing cardiac mechanical performance. Due to its high lipophilicity, it can effectively accumulate in membranes, resulting in isolated coronary arteries maintaining their vasorelaxant effects even after the cessation of the treatment. The results indicate that the cardioprotective effects of benidipine can be augmented through its sustained absorption by cardiac membranes. In isolated rat cardiac tissue, benidipine exhibits protective properties against $H2O_2$-induced injury, indicating that its cardioprotective benefits arise from mechanisms beyond calcium antagonism, including antioxidant activity.

EFFECT ON KIDNEY

Chronic renal failure (CRF) is an unavoidable prelude to end-stage renal disease. The cell cycle is contingent upon delayed rectifier Kv1.3 channels, predominantly located in leukocyte plasma membranes. Benidipine can efficiently block Kv1.3 channels. Benidipine possesses pharmacological effects that impede the advancement of renal fibrosis due to its overexpression in leukocytes during severe chronic renal failure. In the kidneys of rats with severe chronic renal impairment, benidipine demonstrated efficacy in diminishing in-situ leukocyte proliferation. This indicates that benidipine directly diminished the immunological response of the kidneys. This indicates that benidipine impedes the advancement of renal fibrosis and positively influences the reduction of in-situ leukocyte proliferation. Moreover, benidipine is recognized for its reno-protective advantages in kidneys affected by glomerular disorders, as it reduces proteinuria and decelerates the advancement of glomerulosclerosis. Benidipine has been shown to dilate both afferent and efferent glomerular arterioles and reduce glomerular hypertension by inhibiting L- and T-type calcium channels in the renal

CC BY NC

vasculature. Significant potential for renal protection exists, as reports suggest that the impact on the T channel is more pronounced than that on the L channel. Rho kinase activity, crucial for podocyte effacement in immune complex-mediated glomerular disease and other renal disorders, has been shown to diminish upon blockade of the T calcium channel. Inhibiting Rho kinase 1 (ROCK1) activity can protect renal function in diabetes by decreasing fibrosis and epithelial-mesenchymal transdifferentiation through T channel blockage. Benidipine inhibits epithelial-mesenchymal transdifferentiation by obstructing Rho kinase activity. The expressions of ROCK1 mRNA and the proteins ROCK1, SMA, and E-cadherin were quantified using PCR and blotting methodologies. Benidipine can drastically reduce NAG activity, lower urine protein and serum creatinine levels, raise E-cadherin expression, decrease p-MYPT1, ROCK1, and -SMA expression, and increase p-MYPT1 and -SMA expression, all without altering blood pressure or blood glucose levels. Benidipine was discovered to partially inhibit epithelial-mesenchymal transdifferentiation (EMT) by reducing ROCK1 activity. Benidipine's capacity to reduce blood pressure is not the sole mechanism by which it inhibits diabetic nephropathy.

The long-term antihypertensive efficacy of the drugs was evidenced by comparing diastolic blood pressure at the recorded intervals to baseline measurements. Benidipine has exhibited antihypertensive effects on hypertension during both morning and evening administration. Alterations in serum creatinine concentrations and the estimated glomerular filtration rate (eGFR), an indicator of renal function, are utilized to evaluate the reno-protective properties of benidipine. Individuals with hypertension may encounter cardiovascular problems if they also have renal illness. The reno-protective characteristics of Benidipine may be attributed to its inhibitory impact on the T-type calcium channel. Benidipine has been shown to dramatically decrease proteinuria. In comparison to other calcium channel blockers, benidipine positively influences renal function, indicating potential therapeutic advantages as an antihypertensive agent with renoprotective characteristics. The reno-protective characteristics of Benidipine may be attributed to its ability to inhibit T-type calcium channels.

EFFECT ON MUSCLE

This study primarily focused on the impact of benidipine on the airway smooth muscle (ASM) of mice, which plays a crucial role in airway constriction and the development of asthma. Multiple assessments were conducted to determine the efficacy of benidipine in relaxing airway smooth muscle (ASM). The tracheas of male BALB/c mice were utilized to assess tissue tension. Precontraction induced by elevated potassium levels (80 mM) was utilized to assess ASM contraction.

Benidipine exhibits a dose-dependent ability to produce relaxation of both high K^+ and ACh-induced precontraction by obstructing Ca2+ influx through non-selective cation channels (NSCCs) and L-type voltage-dependent Ca2+ channels (LVDCCs). The study indicates that benidipine's prolonged and irreversible effects render it a good option for potential medical applications in the treatment of airway hyper-responsiveness linked to asthma.

EFFECT ON HYPEREMIA PATIENTS

In individuals with essential hypertension, benidipine diminishes forearm reactive hyperemia. Enhancing endothelial function may be necessary to mitigate cardiovascular morbidity.

Calcium channel blockers provide anti-endothelial properties. This clinical study investigated the effects of benidipine, a calcium antagonist, on individuals with hypertension, a condition that leads to endothelial cell dysfunction. Endothelial cells have demonstrated a favorable preventive reaction to benidipine. Benidipine markedly decreased both systolic and diastolic blood pressure during simultaneous evaluation of blood flow response. Following an eight-week regimen of benidipine, the reactive hyperemia ratio exhibited a significant rise. The current study indicates that Benidipine enhances endothelial function as assessed by strain-gauge plethysmography. While it is presumed that reduced blood pressure aids this action, benidipine may exert a direct effect independent of blood pressure reduction. Ultimately, research indicated that benidipine directly influenced endothelial dysfunction induced by hypertension. Due to HGF's protective role against endothelial damage, increased circulating levels of HGF induced by benidipine may enhance endothelial dysfunction.

IN VITRO

EFFECT ON HEART

In atherosclerosis, foam cells are formed when monocyte-derived macrophages infiltrate the arterial wall and accumulate cholesterol. Vascular cell adhesion molecule-1 (VCAM-1, CD104), intracellular cell adhesion molecule-1 (ICAM-1, CD54), and chemokines such as interleukin-8 and monocyte chemoattractant protein-1 (MCP-1) are elevated in atherosclerotic plaques. Benidipine possesses antioxidative properties.

To examine the effect of benidipine on the production of chemokines and surface adhesion molecules in human aortic endothelial cells (HAECs), a 24-hour pretreatment with benidipine markedly diminished the adherence of THP-1 cells to HAECs induced by TNF-α or interleukin-1β. Benidipine significantly diminished the synthesis of MCP-1 and IL-8 by HAECs, while only slightly influencing the expression of ICAM-1 mRNA. Additionally, Benidipine markedly inhibited the elevation of VCAM-1 mRNA in a concentration-dependent manner. Western blot analysis of inhibitory nuclear factor-κB (IκB) phosphorylation and luciferase reporter assays indicated that benidipine suppressed the redox-sensitive transcriptional nuclear factor-κB (NF-κB) pathway. Benidipine may possess anti-inflammatory properties and offer therapeutic or preventive benefits for hypertension and atherosclerosis.

EFFECT ON BONE

Hypertension continues to be a significant health issue, even among older persons predisposed to specific bone-related degenerative disorders. Prevalent conditions affecting a substantial segment of the elderly population include hip fractures and osteoporosis, both marked by skeletal instability and bone deterioration. Research indicates that irregularities in bone mass correlate with elevated blood pressure. Consequently, antihypertensive medications have been regarded as beneficial for concurrently enhancing osteogenesis and modifying bone health. MC3T3-E1 cells were cultured in a humidified incubator at 37 °C with 5% CO2, utilizing minimal medium augmented with 10% fetal bovine serum (FBS; Gibco) and 100 U/ml streptomycin/penicillin (Sigma-Aldrich). Cells were passaged using 0.25% trypsin (Sigma-Aldrich) upon reaching 80% confluence, and the culture media was refreshed every three days. Five × 10^3 MC3T3-E1 cells were introduced into each well of 96-well plates, which were subsequently incubated for 12 hours. The final concentrations of the BD solution were included.

Blank wells and negative controls were established utilizing untreated cells.

The MTT and cell cycle analyses demonstrate that benidipine significantly and dose-dependently enhances the proliferation of MC3T3-E1 cells, thereby laying the groundwork for subsequent osteogenic differentiation. In vitro, benidipine was shown to limit the functionality of osteoclasts. Benidipine enhances the maturation, osteogenic differentiation, and proliferation of mice pre-osteoblasts. A well designed drug delivery mechanism is essential for pure benidipine to enhance osteogenesis in localized applications.

EFFECT ON URETER:

The impact of Benidipine on the contractility of the proximal and distal caprine ureter. The goat ureter was utilized due to its physiological and anatomical similarity to the human ureter. Following the division of the ureters into proximal and distal parts, the serosa was excised. The segments were subsequently partitioned into smaller strips and preserved in an organ bath containing mammalian Ringer solution at 40 °C. A stock solution of benidipine was shown to suppress ureteral activity in vitro, exhibiting a more pronounced impact in the distal ureter compared to the proximal ureter. The study elucidated the impact of benidipine on ureter contractility in relation to dosage for the first time. Benidipine suppressed distal ureter contractility at reduced dosages due to the higher concentration of L-type calcium channels in the lower ureter. Research suggests that Benidipine may be effective in the treatment of ureteric stones, especially those located in the distal ureter.

3. CONCLUSION

Benidipine, a long-acting calcium channel blocker, is utilized for the management of hypertension. In addition to its antihypertensive properties, benidipine exerts other effects on animals. It illustrates that benidipine is a therapeutically efficacious agent with a significant inhibitory impact on distal ureteric contractility, a protective influence on diabetic nephropathy, a decrease in glomerular hypertension, and an effect on osteogenesis and osteoporosis. Given that benidipine is a newly created pharmaceutical, further investigation is necessary to ascertain its effects on both humans and animals.

REFERENCES

1. Oparil, S., Acelajado, M. C., Bakris, G. L., Berlowitz, D. R., Cífková, R., Dominiczak, A. F., Grassi, G., Jordan, J., Poulter, N. R., Rodgers, A., & Whelton, P. K. (2018). Hypertension. Nature Reviews Disease Primers, 4, 18014. https://doi.org/10.1038/nrdp.2018.14
2. Yao, K., Nagashima, K., & Miki, H. (2006). Pharmacological, pharmacokinetic, and clinical properties of benidipine hydrochloride, a novel, long-acting calcium channel blocker. Journal of Pharmacological Sciences, 100(4), 243–261. https://doi.org/10.1254/jphs.dtj05001x
3. Koçak, M. N., Arslan, R., Albayrak, A., Tekin, E., Bayraktar, M., Çelik, M., Kaya, Z., Bekmez, H., & Tavaci, T. (2021). An antihypertensive agent benidipine is an effective neuroprotective and antiepileptic agent: an experimental rat study. Neurological Research, 43(12), 1069–1080. https://doi.org/10.1080/01616412.2021.1949685
4. Yang, Y., Chen, W., Wen, N., Cai, C., Liu, Q. H., & Shen, J. (2019). Benidipine, an antihypertensive drug, relaxes mouse airway smooth muscle. Life Sciences, 227, 74–81. https://doi.org/10.1016/j.lfs.2019.04.036

5. Akinaga, S., Kobayashi, H., Kobayashi, S., Inoue, A., Nakamizo, N., & Oka, T. (1988). Determination of the calcium antagonist benidipine hydrochloride in plasma by sensitive radioimmunoassay. Arzneimittel-Forschung, 38(11A), 1738–1741.
6. Matsunaka, K., Imai, M., Sanda, K., Yasunami, N., Furuhashi, A., Atsuta, I., Wada, H., & Ayukawa, Y. (2022). Therapeutic effect of benidipine on medication-related osteonecrosis of the jaw. Pharmaceuticals (Basel), 15(8), 1020. https://doi.org/10.3390/ph15081020
7. Ma, Z. P., Liao, J. C., Zhao, C., & Cai, D. Z. (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
8. Cakir, T., Yucetas, S. C., Yazici, G. N., Sunar, M., Arslan, Y. K., & Suleyman, H. (2021). Effects of benidipine hydrochloride on ischemia reperfusion injury of rat brain. Turkish Neurosurgery, 31(3), 310–317. https://doi.org/10.5137/1019-5149.JTN.27372-19.3
9. Liu, H. R., Gao, F., Tao, L., Yan, W. L., Gao, E., Christopher, T. A., Lopez, B. L., Hu, A., & Ma, X. L. (2004). Antiapoptotic mechanisms of benidipine in the ischemic/reperfused heart. British Journal of Pharmacology, 142(4), 627–634. https://doi.org/10.1038/sj.bjp.0705847
10. Yao, K., Ina, Y., Sonoda, R., Nagashima, K., Ohmori, K., & Ohno, T. (2003). Protective effects of benidipine on hydrogen peroxide-induced injury in rat isolated hearts. Journal of Pharmacy and Pharmacology, 55(1), 109–114. https://doi.org/10.1111/j.2042-7158.2003.tb02440.x
11. Kazama, I., Baba, A., Matsubara, M., Endo, Y., Toyama, H., & Ejima, Y. (2014). Benidipine suppresses in situ proliferation of leukocytes and slows the progression of renal fibrosis in rat kidneys with advanced chronic renal failure. Nephron Experimental Nephrology, 128(1–2), 67–79. https://doi.org/10.1159/000368080
12. Wu, G., Xu, M., Xu, K., & Hu, Y. (2013). Benidipine protects kidney through inhibiting ROCK1 activity and reducing the epithelium-mesenchymal transdifferentiation in type 1 diabetic rats. Journal of Diabetes Research, 2013, 174526. https://doi.org/10.1155/2013/174526
13. Abe, M., Okada, K., Maruyama, N., Matsumoto, S., Maruyama, T., Fujita, T., Matsumoto, K., & Soma, M. (2011). Benidipine reduces albuminuria and plasma aldosterone in mild-to-moderate stage chronic kidney disease with albuminuria. Hypertension Research, 34(2), 268–273. https://doi.org/10.1038/hr.2010.221
14. Ohta, M., Sugawara, S., Sato, N., Kuriyama, C., Hoshino, C., & Kikuchi, A. (2009). Effects of benidipine, a long-acting T-type calcium channel blocker, on home blood pressure and renal function in patients with essential hypertension: a retrospective, “real-world” comparison with amlodipine. Clinical Drug Investigation, 29(11), 739–746. https://doi.org/10.2165/11320000-000000000-00000
15. Makino, H., Aoki, M., Hashiya, N., Yamasaki, K., Shimizu, H., Miwa, K., Ogihara, T., & Morishita, R. (2005). A calcium-channel blocker, benidipine, improves forearm reactive hyperemia in patients with essential hypertension. Blood Pressure Supplement, 1, 39–44. https://doi.org/10.1080/08038020510040612
16. Matsubara, M., & Hasegawa, K. (2004). Effects of benidipine, a dihydropyridine-Ca^{2+} channel blocker, on expression of cytokine-induced adhesion molecules and

chemoattractants in human aortic endothelial cells. European Journal of Pharmacology, 498(1–3), 303–314. https://doi.org/10.1016/j.ejphar.2004.07.086

17. Wang, B., Yang, J., Fan, L., Wang, Y., Zhang, C., & Wang, H. (2021). Osteogenic effects of antihypertensive drug benidipine on mouse MC3T3-E1 cells in vitro. Journal of Zhejiang University Science B, 22(5), 410–420. https://doi.org/10.1631/jzus.B2000628
18. Mathew, S. K., Naik, G. S., & Peedicayil, J. (2017). Inhibition by benidipine of contractility of isolated proximal and distal caprine ureter. International Journal of Applied & Basic Medical Research, 7(3), 155–159. https://doi.org/10.4103/ijabmr.IJABMR_87_16