COMPARISON OF VASCULAR CALCIUM CHANNEL BLOCK PROMOTED BY VERAPAMIL AND NIFEDIPINE BY MOLECULAR MODELING

Comparação do bloqueio de canal de cálcio vascular promovido por verapamil e nifedipina por modelagem molecular

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ABSTRACT

Cardiovascular diseases are extensively studied due to their risks and global prevalence. Thus, searching for new treatments is an ongoing process, and plant-derived compounds are a promising source of drugs. Nowadays, molecular modeling analyses are conducted to understand the structure-function relationship of a pharmacological target and its protein-ligand interactions (i.e., the exact mechanism of pharmacological action). This study aimed to compare the voltage-dependent calcium channel (CaV) blockade promoted by verapamil and nifedipine using molecular modeling analyses. Tests and analyses were performed using the Dockthor and Chimera programs, respectively. The t-test was used to compare the affinity energies. Data analysis occurred in the GraphPad Prism software; statistical significance was set at $p < 0.05$. Verapamil had a higher affinity than nifedipine ($p < 0.0001$). Although the drugs presented different binding sites, they successfully blocked the CaV and prevented the calcium influx into the cell. These findings could be useful for the prospect of new drugs that block CaV.

Keywords: Antihypertensive agents; calcium channel blocker; docking molecular.

RESUMO

As doenças cardiovasculares são extensamente estudadas devido aos seus riscos e prevalência mundial. Sendo assim, a pesquisa por novos tratamentos é um processo contínuo e as substâncias isoladas de plantas são uma fonte promissora de fármacos. Atualmente, análises de modelagem molecular são realizadas para compreender a relação entre estrutura-função de um alvo farmacológico e suas interações proteína-ligante, e assim o mecanismo exato da ação farmacológica. Diante disso, este trabalho visou comparar o bloqueio do canal de cálcio dependente de voltagem promovido por verapamil e nifedipina com análises de modelagem molecular. Os experimentos de *docking* molecular foram realizados usando o portal DockThor e análises com o programa Chimera. As energias de afinidade foram comparadas utilizando o programa GraphPad Prism, com o teste "t", em que valores de $p < 0,05$ foram considerados significantes. Os resultados mostram que o verapamil teve uma afinidade maior do que a de nifedipina ($p < 0,0001$). As simulações de configuração no canal foram diferentes, cada uma bloqueando o seu poro, o que impede o influxo de cálcio na célula. Estes dados serão úteis para comparar à prospecção de novas drogas que bloqueiam o canal de cálcio.

Palavras-chave: Agentes anti-hipertensivos; bloqueador de canal de cálcio; modelagem molecular.

INTRODUCTION

Cardiovascular diseases are extensively studied due to their risks and global prevalence. Although their incidence is decreasing in Brazil, they are still the leading cause of death.[1] Thus, searching for new treatments is an ongoing process, and plant-derived compounds are a promising source of drugs.[2]

Molecular modeling analyses are conducted to understand the structure-function relationship of a pharmacological target and its protein-ligand interactions.[3-6] These analyses provide information about the structural dynamics of the molecules and also the energy required to bind them in a target protein, which is essential for characterizing the mechanism of drug action.[7]

In blood pressure regulation, the α-1C subunit of L-type Ca^{2+} channels (CaV1.2) is the main pathway for Ca^{2+} influx into cells. Upon activation, these channels mediate Ca^{2+} into the cytoplasm, triggering vasoconstriction.[8-10] Voltage-gated calcium channel (CaV) blockers are commonly used for treating hypertension and are classified as dihydropyridines or non-dihydropyridines.[11-13] Dihydropyridines (e.g., amlodipine, nifedipine, and felodipine) cause a predominant vasodilatory effect with minimal interference in heart rate and systolic function, which justifies their use as antihypertensive drugs. Non-dihydropyridines (e.g., phenylalkylamines [verapamil] and benzothiazepines [diltiazem]) have a less pronounced vasodilatory effect and act on the cardiac muscle and the cardiac conduction system. Therefore, they reduce heart rate, exert antiarrhythmic effects, and may depress systolic function.[14] Thus, this study aimed to compare the CaV blockade promoted by verapamil and nifedipine using molecular modeling analyses.

MATERIAL AND METHODS

This study was quantitative and experimental. Verapamil (CID 2520, Figure 1A) and nifedipine (CID 4485, Figure 1B) were obtained from the PubChem database.[15] The homologous structure of CaV (ID 4MS2) was obtained from the Protein Data Bank (Figure 1C).

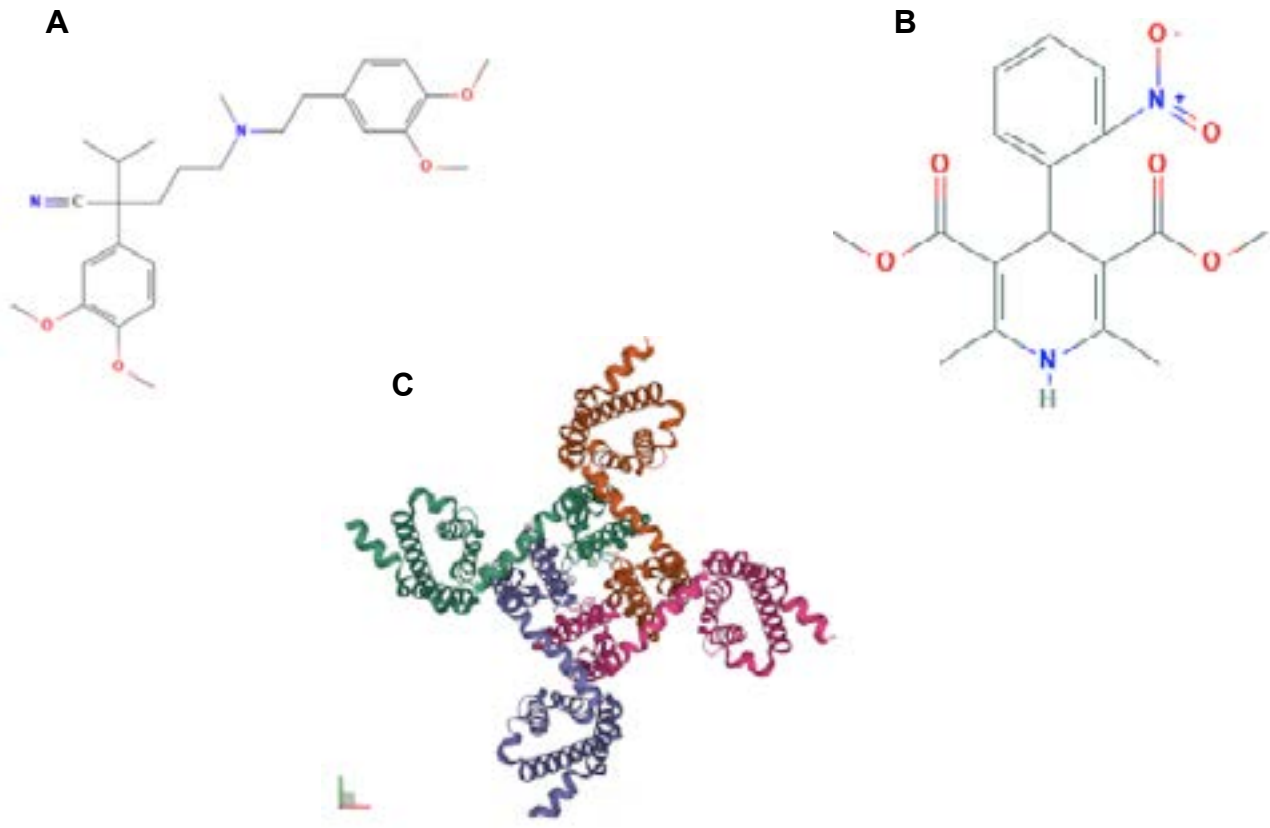

Figure 1. Molecular structure of verapamil (**A**, C27H38N2O4), nifedipine (**B**, C17H18N2O6) and the 3D structure of CaV (**C**).

Molecular modeling experiments were conducted using the online portal DockThor[16]; results were ranked by their highest affinity with the CaV protein. The three best docking configurations with the highest affinity for the target were selected for further analyses, and the positions were presented using the Chimera software (version 1.14).

Affinity energies were analyzed using the GraphPad Prism software, employing the t-test to compare the results of verapamil and nifedipine. Statistical significance was set at $p < 0.05$.

RESULTS

One million docking positions were generated for the drugs (verapamil and nifedipine) against the CaV protein (ID 4MS2). The three best positions based on binding energy values are presented in Table 1. Lower binding energy indicates a more stable interaction as a result of the hydrophobic and electrostatic bonds between the molecules and the CaV protein. Notably, verapamil demonstrated a significantly higher affinity for CaV than nifedipine.

Table 1. Binding affinities of drugs in the CaV channel.

Drug	**Affinity** (kcal/mol) Mean ± SEM
Verapamil	-9.696 ± 0.08*
Nifedipina	- 8.031 ± 0.009

Kcal/mol: kilocalorie per mole; SEM: standard error of the mean; *p < 0.0001 (verapamil vs. nifedipine), t-test

Configuration analysis of the drugs in the 3D structure of the CaV revealed that they occupy similar positions in the pore region. However, verapamil and nifedipine bind to different amino acid residues (Figure 2). Both drugs blocked the channel pore, hampering the influx of Ca^{2+} into the cells.

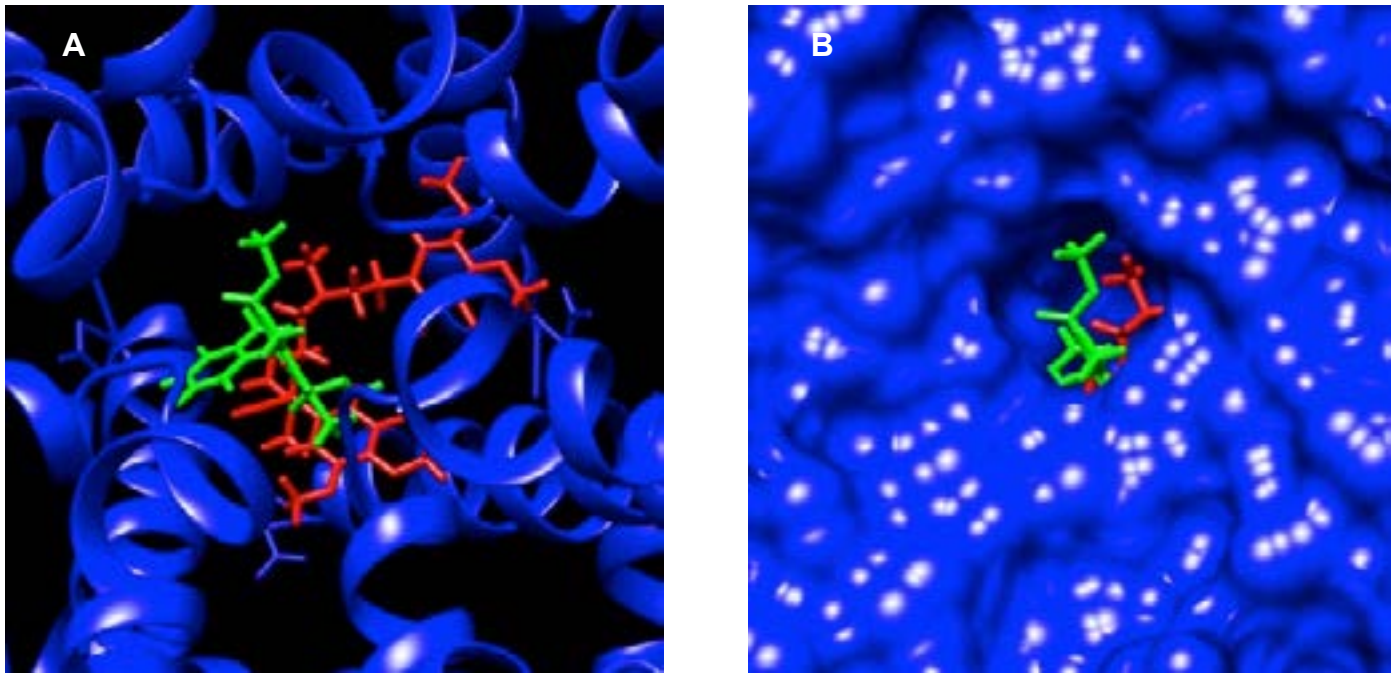

Figure 2. Positioning of verapamil (red) and nifedipine (green) in the 3D structure of the CaV channel (blue). **A:** quaternary protein structure; **B:** hydrophobic protein structure.

DISCUSSION

The CaV contains a large subunit (α1) that forms the pore using four homologous domains, each with six transmembrane segments and a loop that forms the pore[17](Figure 1C).

Phenylalkylamine-type CaV blockers (verapamil; Figure 1A) and benzothiazepines (diltiazem) are used for treating atrial arrhythmias.[18] Different affinities were observed for verapamil and nifedipine (Table 1); the former showed higher affinity. Verapamil presents a longer carbon chain than nifedipine, which allows more interactions with the amino acid residues of the CaV pore; this fact may justify the higher affinity. Phenylalkylamines have two aromatic rings linked by a central aliphatic chain, containing a tertiary amino group that can enter the external opening of the pore via the open activation gate and bind to a specific receptor site in a positively charged protonated form.[18,19]

Conversely, dihydropyridines (nifedipine; Figure 1B) interact with the S6 segments in domains III and IV, leading to a proposed interface-domain model for drug binding and action.[17,20] Another molecular modeling study suggests that these amino acid residues are located on the luminal side of the pore and collaborate to form the drug-binding site.[21]

Other dihydropyridine CaV blockers (nimodipine, benidipine, and amlodipine) have been evaluated for their orientation in blocking L-type calcium channels (CaV1.2).[22] Results similar to those of nifedipine (Figure 2) were obtained with uncarilan A, an alkaloid isolated from *Uncaria rhynchophylla*, a plant used in traditional Chinese medicine. The molecular simulation showed that uncarilan A inhibited the CaV, preventing calcium influx.[23]

This study used molecular modeling analyses to determine the CaV blockade promoted by verapamil and nifedipine as standard blockers used in clinical practice. Findings will serve to compare with new drugs that have potential CaV-blocking effects.

CONCLUSION

Nifedipine and verapamil blocked the CaV using different binding sites in the channel pore; the latter had a higher affinity. These findings will aid in the evaluation of new drugs that may also target and block the CaV.

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