World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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Predicting L-carnitine potential as LDH-inhibitor through *In Silico* approach

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World Journal of Advanced Research and Reviews, 2024, 21(02), 481–486

Publication history: Received on 25 December 2023; revised on 03 February 2024; accepted on 06 February 2024

Article DOI: https://doi.org/10.30574/wjarr.2024.21.2.0446

Abstract

Background: L-carnitine (LC) is suggested to inhibit lactate accumulation post-exercise. However, the exact mechanism of how LC inhibits lactate accumulation is still elusive. Lactate dehydrogenase (LDH) is a key enzyme that catalyzes the interconversion of pyruvate and lactate. Thus, the present study aimed to investigate the effect of LC in inhibiting LDH through in silico approach.

Methods: The methods used in this study consisted of protein and ligand data collection, pharmacokinetic analysis, molecular docking, and molecular dynamics. The ligands used in this study are L-Carnitine and LDH-A inhibitor (AZ-33) as the control ligand, while the protein is human muscle LDH-A.

Results: The results of this study indicate that L-carnitine has the potential to be an oral-drug candidate as it fulfills all Lipinski criteria and shows good cell membrane permeability. However, molecular docking showed that LC has weaker binding affinity values (-5.2 kcal/mol) than AZ-33 (-8.8 kcal/mol) to LDH-A. LC also interacts with different amino acid residues in LDH-A compared to AZ-33. Despite that, the molecular dynamic study revealed that LC forms more stable interactions with LDH-A.

Conclusion: LC can bind to LDH-A with more stability than AZ-33, despite its weaker binding affinity to LDH-A and different interacting residues. Further study is needed to investigate the other mechanism that explains the effect of LC in inhibiting lactate accumulation.

Keywords: Carnitine; Lactate Dehydrogenase; In Silico; Molecular Docking

1. Introduction

Dietary supplement consumption is prevalent in athletes. This is mainly due to several claimed benefits, including supporting their well-being and enhancing their athletic performance (1). It has been reported that around 60% of athletes have taken dietary supplement at least once (2). Recently, the use of amino acid and/or protein supplementation is gaining interest, such as L-Carnitine (LC) (3). L-Carnitine (LC) is a naturally occurring quaternary amine which is predominantly stored in skeletal muscle and the heart (4). LC can be produced internally through the methylation of the essential amino acid lysine, however, only 25% of adults' daily LC needs are met through endogenous biosynthesis (5). Consequently, supplementation of LC, either through specific dietary sources or as a nutritional supplement, becomes necessary, particularly for athletes(6).

LC plays a crucial role in facilitating the mitochondrial membrane transport of acyl-coenzyme A (CoA), thereby promoting fatty acid oxidation process necessary for ATP generation during exercise (7). Furthermore, LC has the

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capacity to reduce the accumulation of blood lactate during exercise, which may ameliorate muscle damage caused by strenuous exercise which is a common problem in athletes (8,9). Lactate dehydrogenase (LDH) facilitates the conversion between pyruvate and lactate (10). Therefore, it holds a critical role in physiological functions such as energy metabolism, glycolysis, and intracellular redox regulation (11). Recently, there has been growing evidence of natural compounds for modulating LDH activity. Modulating LDH has shown the potential to induce beneficial effects, such as anti-oxidative stress, anti-inflammatory, and anti-apoptotic (12). However, whether LC able to inhibit LDH activity is still unknown.

Considering its essential role in energy metabolism, the supplementation of L-carnitine is suggested to have beneficial effects on ameliorating exercise-induced muscle injury related to lactate accumulation (9,13,14). However, the exact mechanism of LC in inhibiting lactate accumulation is still elusive. Thus, the present study aimed to investigate the effect of LC in inhibiting LDH activity by an in silico approach. The reliability of the binding affinity between LC and LDH was further validated by molecular dynamics simulation.

2. Material and Methods

2.1. Predicting pharmacokinetic property

The SwissADME (http://www.swissadme.ch/) database was used to predict the absorption, distribution, metabolism, and excretion (ADME) characteristics of LC by submitting its canonical smile obtained from the PubChem database (PubChemID 10917). In order to assess the drug-likeliness of LC, several properties were considered, namely molecular weight, oral bioavailability, predicted central nervous system activity, predicted aqueous solubility Lipinski rule of 5, and predicted human oral absorption. A compound is considered to be a good drug candidates if it does not violate the Lipinski rule, has five or fewer hydrogen bond donors, ten or fewer hydrogen bond acceptors, and a molecular mass of 500 kg/mol or less (15).

2.2. Molecular Docking

The 3D structure of L-carnitine (PubChemID 10917) was obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/). The control ligand chosen in this study is an established LDH inhibitor, namely AZ-33 (PubChemID 56844254). The tested ligand compounds were further analyzed for their pharmacokinetic profiles using SwissADME (http://www.swissadme.ch/index.php) by entering the SMILES formula of each active substance. Each compound underwent Lipinski's Rule of Five analysis to determine the suitability of oral-administered drug.

LDH is an enzyme complex composed of four subunits, including two distinct isoforms: LDH-A and LDH-B. LDH-A holds a significant position in the glycolytic pathway owing to its heightened affinity for pyruvate. It functions by catalyzing the conversion of pyruvate to lactate while oxidizing NADH to NAD+ (10). Therefore, present study chose human muscle LDH-A as the target protein. Crystal structure of LDH-A (PDB ID: 1I10) was downloaded from Protein Data Bank (www.rscb.org) and optimized by removing water molecules and adding hydrogen atoms in PyMol software 2.5. Ligand compound's energy was minimized using OpenBabel in order to achieve optimum binding strength. Each compound was then docked specifically with LDH using PyRx 0.9.5 software on a grid of 50 x 50 x 50 Å (x, y, z) with grid box center at X = 16.237, Y = -2.233, and Z = 129.748. Ligand and target protein complexes were subsequently visualized and analyzed for their interaction using the Discovery Studio 2021 software.

2.3. Molecular Dynamics Study

Molecular dynamics simulations were conducted to assess the stability of the interaction between LC and LDH. Control ligands also underwent identical simulation as a comparation. Initially, LC was docked with LDH using PyRx software and the obtained structures were saved in PDB format. These structures were then submitted to CABS-flex 2.0, a web-based protein structure modeling tool (http://biocomp.chem.uw.edu.pl/CABSflex2) developed by Kmiecik et al. (16). The simulation involved 50 cycles, and the A chain was selected for analysis. Default web server settings were applied for the simulation, and the root mean-square fluctuation (RMSF) value was calculated. A higher RMSF value indicates greater flexibility (15).

3. Results and Discussion

3.1. Pharmacokinetic Analysis

SwissADME was used to analyze pharmacokinetic profiles of LC. The oral regiment's absorption capacity and permeability are assessed using Lipinski's 'Rule of Five' criterion. Our findings as shown in Table 1 indicated that LC as well as control ligands have met Lipinski criteria without any violation. An orally active drug-like compound should not have more than one violation of these criteria: hydrogen bond donors not greater than 5, hydrogen bond acceptors not greater than 10, molecular weight not greater than 500 Da, and octanol-water partition coefficient (log P) not greater than 5. LC has molecular weights of 161.20 Da, AlogP o/w value of -2.40, one hydrogen bond donor, and three hydrogen bond acceptors. Either LC and AZ-33 obeys all Lipinski's rules which indicates its drug-like properties. Moreover, the predicted aqueous solubility (logS) of LC was 0.05, meaning that it is highly water-soluble. Meanwhile, AZ-33 has logS score of -6.32 which indicates its low solubility in water and thus low GI absorption. On the other hand, LC has acceptable oral bioavailability of 55%, while AZ-33 only 11%. LC also has topological polar surface area (TPSA) value of less than 140 angstroms which indicates that it is considered as a small molecule, thus it is potentially cell-membrane permeable (17). Therefore, based on these data, LC is suitable for oral route administration and it shows good cell-membrane permeability, which may permits its interaction with intracellular enzymes, such as LDH.

Table 1 Pharmacokinetics Profiles of L-Carnitine

Compound	Mol. Weight (Da)	H Bond Donor	H Bond Acceptor	Log P	Log S	TPSA (Å)	Bioavailability Score	Lipinski Criteria Violation
L-Carnitine	161.20	1	3	-2.40	0.05	60.36	0.55	0
AZ-33 (Control)	497.56	4	7	3.02	-6.32	173.93	0.11	0

3.2. Molecular Docking

LC supplementation has been reported to reduce muscle damage related to exercise associated with increased lactate accumulation (9,13,14). LC supplementation resulted in lower lactate level after both aerobic and anaerobic exercise in gymnast athletes (18). This effect is hypothesized due to the role of LC in maintaining the acetyl CoA/CoA ratio, thereby allowing continuous pyruvate dehydrogenase activity and preventing lactate formation (19). Therefore, it is logical to predict that LC may inhibit LDH and subsequently prevents its enzymatic activity. However our molecular docking simulation showed otherwise. The binding affinity score of LC and LDH-A was only -5.2 kcal/mol, while AZ-33 was -8.8 kcal/mol (Table 1). The binding affinity values of ligands and proteins can be interpreted as the average free energy of the conformations of all interacting molecules. The lower the binding affinity correlates with higher possibility to form a bond (20). The interaction of LC and AZ-33 to LDH-A amino acid residues are depicted by 2D visualization using Discovery Studio (Figure 1). There is no similar bond observed between LC and AZ-33 to LDH-A which indicates that LC bind to different domain in LDH-A compared to AZ-33.

Table 2 Molecular Docking Result of L-Carnitine and AZ-33 (Control) to LDH-A

Compound	Binding Affinity (Kcal/mol)	Interaction with Amino Acid	
		Hydrophobic Bond	Hydrogen Bond
L-carnitine	-5.2	-	GLY96, ASN137, THR247
AZ-33 (control)	-8.8	ASP257, ARG268, LEU69, LWU182	LYS41, ARG72, ARG170, VAL269

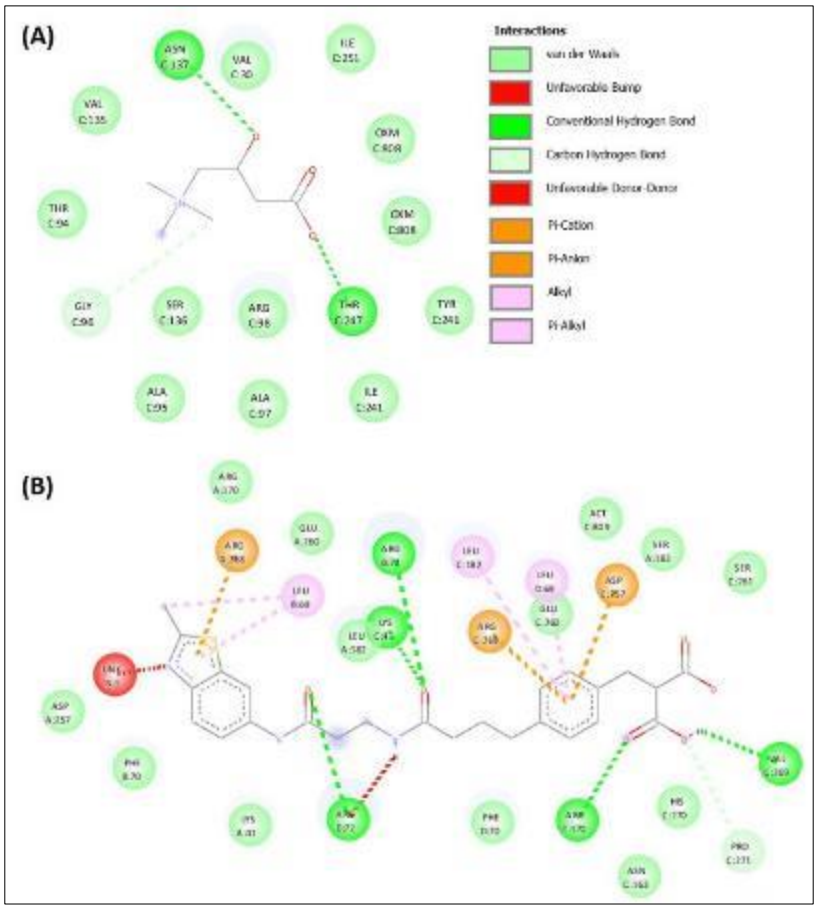

Figure 1 2D Interaction of selected ligand with LDH-A. (A) L-carnitine, (B) AZ-33

3.3. Molecular Dynamics

The molecular dynamic results show that the ligands used in this study form stable bonds to the target proteins (in this case, LDH-A). Based on the plot (Figure 2), it can be observed that several residues underwent fluctuations. The structure is considered to be stable if the fluctuation of RMSF value is in the range of 1-3 Å. However, higher peaks indicates high fluctuation rates and significant conformational changes are anticipated (21). LC and LDH-A interaction showed milder fluctuations than AZ-33 and LDH-A. Therefore, based on the overall results of fluctuations, it can be concluded that LC have more stable interactions with LDH-A, despite the binding affinity is not as strong as AZ-33.

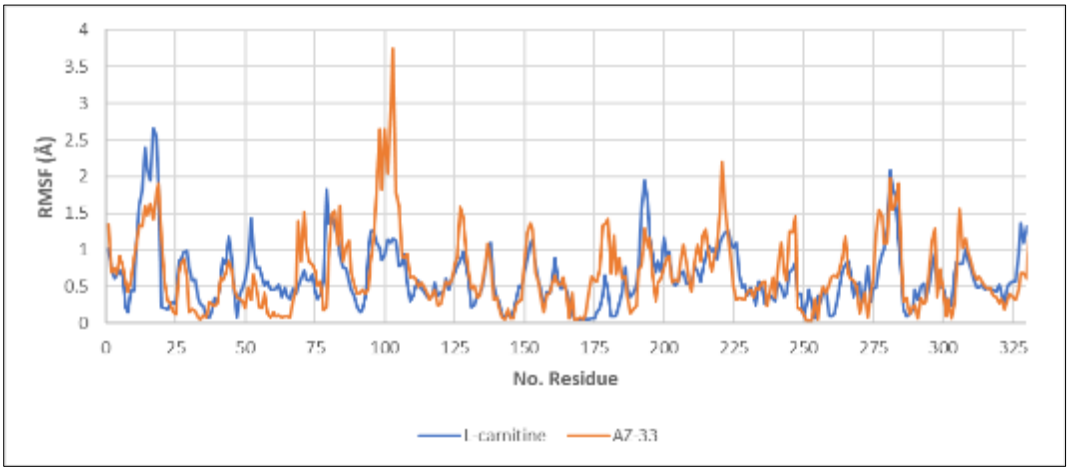

Figure 2 RMSF value of molecular dynamics simulation between LC with LDH compared to control ligand

4. Conclusion

Molecular docking and molecular dynamics simulation revealed that LC bind to LDH-A with weaker affinity than AZ-33 and interact with different domain within LDH-A, but more stable interactions. Despite that, LC still possess good potential as drug-like compound because of its physicochemical properties and is suitable for oral administration, unlike AZ-33. Although our results do not support the inhibitory activity of LC to LDH-A, it does not deny the fact that LC supplementation can decrease lactate accumulation as reported in previous clinical studies. Therefore, based on our study, LC does not inhibit LDH the same way as the developed LDH-inhibitor drug (in this case, AZ-33). Further study is needed to investigate the other mechanism which explain the effect of LC in inhibiting lactate accumulation.

Compliance with ethical standards

Acknowledgement

The authors are grateful of the support from the Faculty of Medicine and the Faculty of Sport Sciences, State University of Malang.

Disclosure of Conflict of Interest

The authors declare no conflict of interest.

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