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Bioinformatics assessment on the potential of Lipoteichoic Acid (LTA) of Lactic Acid Bacteria (LAB) as topical therapy for inflammatory skin diseases

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ABSTRACT

Background: Lipoteichoic Acid (LTA) of Lactic Acid Bacteria (LAB) is a cell wall component of LAB with immunomodulatory properties towards inflammatory skin diseases due to immune dysregulation, such as atopic dermatitis. Topical glucocorticoids are given as the mainstay therapy in some inflammatory skin diseases. However, the adverse effects of topical glucocorticoids are commonly found, such as skin atrophy, reduced pigmentation, and masking-effect of skin infections. Therefore, safer therapy for inflammatory skin diseases is necessary to be developed. This study aimed to analyze the potential of LTA of LAB against glucocorticoid receptors (GR) as a topical therapy for inflammatory skin diseases.

Methods: This study used the bioinformatics method with molecular docking to predict the binding site and the binding affinity of LTA of LAB to GR and analyze the toxicity of LTA of LAB. According to the previous studies, this research was also conducted within *silico*/ molecular docking. Data were analyzed using *Toxtree* v 2. 6.13 application to evaluate the skin irritation.

Results: LAB's lipoteichoic acid (LTA) has the same binding site with glucocorticoid control compounds to the GR. Toxicity analysis shows that LTA of LAB is not irritative to the skin.

Conclusion: LAB's lipoteichoic acid (LTA) is potential as alternative topical therapy for inflammatory skin diseases.

Keywords: Lipoteichoic acid of lactic acid bacteria, glucocorticoid receptors, bioinformatics, inflammatory skin diseases.
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INTRODUCTION

Lipoteichoic acid (LTA) is a structural component found in Gram-positive bacteria's cell walls that play important roles in the growth and physiology of the bacteria and is an immunostimulant component of pathogenic and non-pathogenic Gram-positive bacteria.[1] Lactic Acid Bacteria (LAB) are Gram-positive bacteria and some strains of *Lactobacillus* have probiotic properties, providing health benefits, and obtained Generally Recognized as Safe (GRAS) status.[2,3] The component of the LAB cell wall, namely Lipoteichoic Acid (LTA), can improve inflammatory skin diseases related to immune dysregulation, such as atopic dermatitis.[1,4]

Some inflammatory skin diseases, such as atopic dermatitis, psoriasis, seborrheic dermatitis, neurodermatitis, nummular eczema, and allergic contact dermatitis, are treated with topical glucocorticoids (corticosteroids) as anti-inflammatory and anti-mitotic agents.[5] The mechanism of glucocorticoid action is mediated by Glucocorticoid Receptors (GR) found in most human body tissues, including the skin and skin appendages.[6,7] Topical glucocorticoids also decrease the connective tissue molecule synthesis.[8] Local adverse effects on the use of topical glucocorticoids that are often found are striae, skin atrophy, perioral dermatitis, acne, rosacea, reduced pigmentation, hypertrichosis, and masking-effect of skin infections.[8,9] Therefore, it is necessary to develop a better alternative for treating inflammatory skin diseases.[5]

Bioinformatics is the application of computer technology for managing data/ biological information. In discovering new drugs using a computer system, the screening method is *in silico* or virtual screening.[10] The *in silico* method with molecular docking can predict and analyze potential bioactive compounds against the binding sites, estimate their binding affinity, and help see the interactions between potential bioactive compounds for therapeutic and healing efforts against disease or inflammation.[4]

In vitro and *in vivo* studies on the anti-inflammatory properties of LTA of LAB have

been conducted. Lipoteichoic Acid (LTA) from *Lactobacillus plantarum* K8 (KCTC10887BP) relieves Tumor Necrosis Factor-α (TNF-α)-induced inflammation through down-regulation of Nuclear Factor-kβ (NF-kβ) in the human colon cell line HT-29 and inhibits TNF-α induced by Lipopolysaccharide (LPS) in mice with endotoxin shock.[11,12] Lipoteichoic acid (LTA) from *Lactobacillus acidophilus* NCFM decreases the pro-inflammatory cytokine Interleukin-12 (IL-12) and TNF-α and increases IL-10 in an animal model of colitis.[13] *In silico, in vitro,* and *in vivo* studies complement each other in the drug design process.[14] Therefore, this study analyzed the potency of LTA of LAB by molecular docking against glucocorticoid receptors (GR) prior to *in vivo* study. A study on LTA of LAB is expected to be an alternative topical therapy for inflammatory skin diseases.

METHODS

This research was conducted within *silico*/ molecular docking according to the previous methods.[15-17] The predictive ligand or compound information was obtained from the PubChem database, accessed at the website (https://pubchem.ncbi.nlm.nih.gov/). The specific website link for lipoteichoic acid as the predictive ligand was (https://pubchem.ncbi.nlm.nih.gov/compound/137349712). The data accessed through this database was the CID of Lipoteichoic Acid (LTA) 137349712 and the canonical smile compound.

The binding receptor or target protein for the LTA ligand was obtained from the SWISS Target Prediction web server (http://www.swisstargetprediction.ch) by inputting the predicted ligand's canonical smile on the web-server, then clicking "*predict the target.*" After that, the prediction result with a list of target proteins and information in a UniProt ID would show up. The UniProt database determined the target protein's function (https://www.uniprot.org/). This information helps determine the relationship of predictive ligand on the disease pathway. The binding receptor of the LTA ligand was Glucocorticoid Receptor (GR) with the ID UniProt P04150.

Control ligands or control compounds are ligands that function against the target protein. The control ligands were obtained from the DrugBank database (https://go.drugbank.com) by inputting the target protein's name in the search column. The binding site of the control ligand to the target protein was compared with the predictive one to determine its role on the target protein. The control ligands in this study were hydrocortisone, clocortolone, triamcinolone, and beclomethasone dipropionate.

The docking preparation was conducted by downloading the 3D structures of predictive ligand (LTA) and control ones (hydrocortisone, clocortolone, triamcinolone, and beclomethasone dipropionate) in the PubChem database. The compounds downloaded from PubChem were still in the form of an SDF file extension and converted into a PDB extension using the PyMol application. The 3D structure of the target protein was downloaded from the Protein Data Bank database (https://www.rcsb.org) using the PDB ID obtained from the UniProt database. The ID for the PDB Glucocorticoid receptor (GR) was 5e69. Before docking, the target protein was cut from water molecules and natural ligands using the PyMol application (https://pymol.en.softonic.com/?ex=DSK-1262.10).

Docking of the compound was done using the PyRx application (https://pyrx.sourceforge.io/downloads). The result of compound docking was the binding affinity value of the compound. The smaller the binding affinity value, the better the compound's affinity for the target protein. The docking compound on PyRx was then stored for visualization.

The docking results were visualized to see the suitability of the predictive compound's site with the control ones against the target protein. Visualization of docking results used two applications, PyMol and the Discovery Studio application. PyMol was used to determine the similarity binding position of predictive and control compounds. Positive docking results with PyMol are shown by the similarity binding position or binding site of the predictive ligand and the control ones. On the other hand, the Discovery Studio application determined the amino acid residues where the compound binds to the target protein. The similarity of the amino acid residues of the predictive ligand and the control ligand's positive docking results with Discovery Studio application. If there are similarities in the amino acid residues of both ligands, then the predictive ligand may have the same function as the control ones. Predicting skin irritation was done with the *Toxtree* v 2. 6.13 application. The prediction was made by inputting the canonical smile of the predictive ligand of LTA into the application.

RESULTS

The molecular docking results in this study consist of visualization of the interaction between predictive ligand, control ligands, and binding receptor, binding affinity value, and predictive analysis of potential irritation to skin.

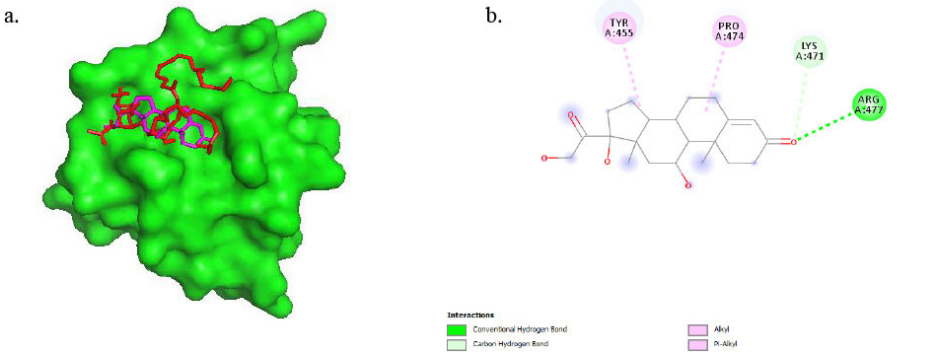

Figure 1. The docking result of LTA and hydrocortisone with GR. (a). 3D Visualization: LTA (red), hydrocortisone (magenta), GR (green) and (b) 2D visualization: amino acid residues in LTA and hydrocortisone.

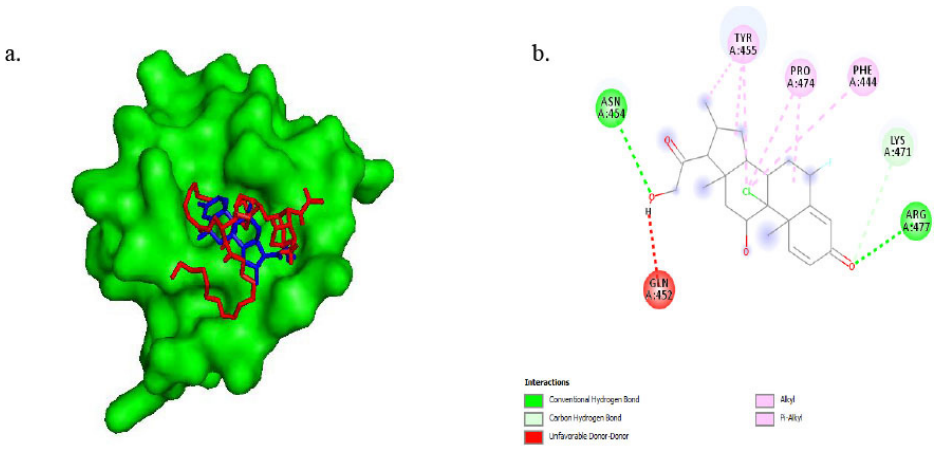

Figure 2. The docking result of LTA and clocortolone with GR; (a) 3D Visualization: LTA (red), clocortolone (blue), GR (green) and (b) 2D visualization: amino acid residues in LTA and clocortolone.

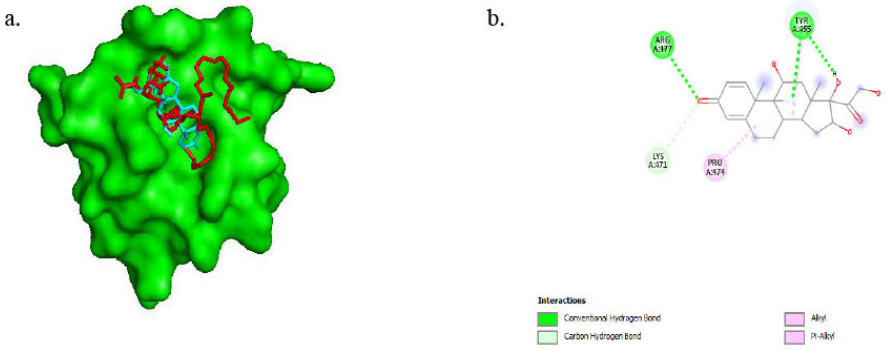

Figure 3. The docking result of LTA and triamcinolone with GR; (a) 3D Visualization: LTA (red), triamcinolone (cyan), GR (green) and (b) 2D visualization: amino acid residues in LTA and triamcinolone.

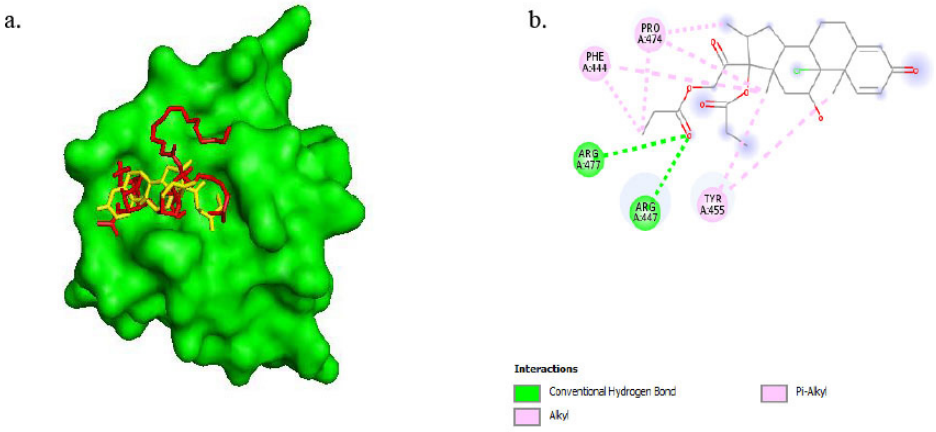

Figure 4. The docking result of LTA and beclomethasone dipropionate with GR; (a) 3D Visualization: LTA (red), beclomethasone dipropionate (yellow), GR (green) and (b) 2D visualization: amino acid residues in LTA and beclomethasone dipropionate.

Visualization of The Interaction between LTA, Hydrocortisone, and GR

The 3D visualization of the interaction of LTA, hydrocortisone, and GR (Figure 1a) shows that LTA and hydrocortisone have the same binding site on GR. The 2D visualization of LTA dan hydrocortisone (Figure 1b) shows the similarity of the amino acid residue TYR A455.

Visualization of the interaction between LTA, clocortolone, and GR

The 3D visualization of LTA, clocortolone and GR interaction (Figure 2a) shows that LTA and clocortolone have the same binding site on GR. The 2D visualization of LTA and clocortolone (Figure 2b) shows the similarity of the amino acid residue TYR A455 and PHE A444.

Visualization of The Interaction between LTA, Triamcinolone, and GR

The 3D visualization of LTA, triamcinolone, and GR interaction (Figure 3a) shows that LTA and triamcinolone have the same binding site on GR. The 2D visualization of LTA dan triamcinolone with GR (Figure 3b) shows the similarity of the amino acid residue TYR A455.

Visualization of The Interaction between LTA, Beclomethasone dipropionate, and GR

The 3D visualization of LTA, beclomethasone dipropionate, and GR interaction (Figure 4a) shows that LTA and beclomethasone dipropionate has the same binding site on GR. The 2D visualization of LTA and beclomethasone (Figure 4b) shows the similarity of the amino acid residue ARG A447, PHE A444, and TYR A455.

Visualization of The Interaction between LTA, Hydrocortisone, Clocortolone, Triamcinolone, Beclomethasone Diproprionate, and GR

The 3D visualization of LTA, hydrocortisone, clocortolone, triamcinolone, beclomethasone dipropionate, and GR interaction (Figure 5a) shows that LTA and the four glucocorticoid control ligands have the same binding site on GR. The 2D visualization of LTA amino acid residues

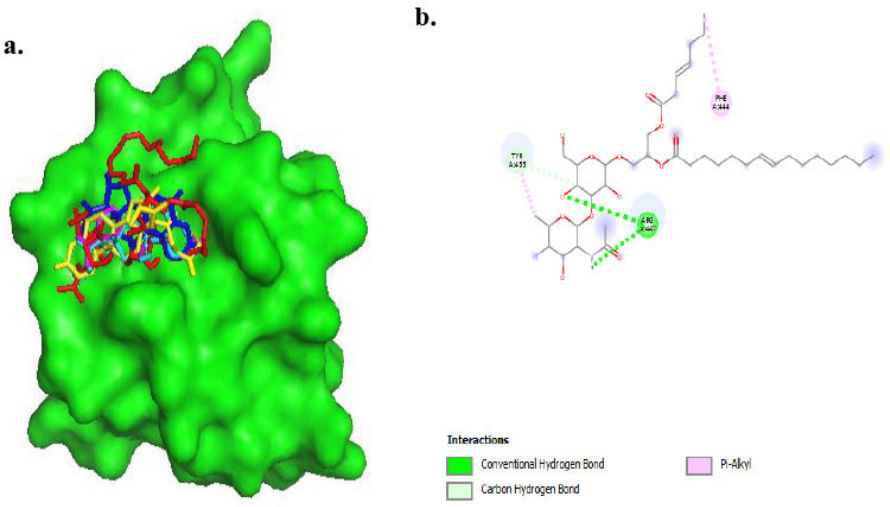

Figure 5. The docking result of LTA, hydrocortisone, clocortolone, triamcinolone, and beclomethasone dipropionate with GR; (a) 3D Visualization: LTA (red), hydrocortisone (magenta), clocortolone (blue), triamcinolone (cyan), beclomethasone dipropionate (yellow), GR (green) and (b) 2D visualization: amino acid residues in LTA and GR.

Table 1. Binding affinity between control compounds and LTA of LAB with GR.

Control and Natural Compounds	Binding Affinity (kcal/ mol)
Hydrocortisone	-6,4
Clocortolone	-6,3
Triamcinolone	-6,9
Beclomethasone dipropionate	-5,8
Lipoteichoic acid of lactic acid bacteria	-4,8

with GR (Figure 5b) shows bonds in the amino acids ARG A447, TYR A455, and PHE A444.

Molecular Docking Analysis based on Binding Affinity Value

Molecular docking was performed with GR's 3D structure with control ligands (hydrocortisone, triamcinolone, clocortolone, and beclomethasone dipropionate) and predictive or natural ligands, which was LTA. The results of molecular docking using PyRx software are shown in Table 1. Table 1 shows that the binding affinity of LTA to GR is the highest among the glucocorticoid control compounds with -4.8 kcal/mol.

Predictive Analysis of Potential Irritation to Skin

Ligand's name is Lipoteichoic acid. Canonicle SMILE: CCCCCCCC=CCCCCCCC(=O)OC(COC1C(C(C(C(O1)CO)O)OC2C(C(C(C(O2)C)N)O)NC(=O)C)O)COC(=O)CCCCCC. The result is not irritative to the skin using *Toxtree* software.

DISCUSSION

The results of LTA docking are in line with *in vivo* and *in vitro* studies on the anti-inflammatory effects of LTA. Lipoteichoic Acid (LTA) from *L. acidophilus* NCFM showed anti-inflammatory activity in a mouse model of dextran sulfate sodium (DSS)-induced colitis by reducing the production of pro-inflammatory cytokines IL-12 TNF-α and enhancing the production of the anti-inflammatory cytokine IL-10.[18] Moreover, LTA of *L. plantarum* K8 (KCTC 10887BP) inhibits viral pathogen-induced inflammatory responses in porcine intestinal epithelial cells due to attenuate phosphorylation of extracellular signal-regulated kinase (ERK), p38 kinase, and activation of NF-kβ, by LTA of *L. plantarum* K8 (KCTC 10887BP), resulting in decreased IL-8 production.[19] The anti-inflammatory ability of LTA is similar to the mechanism of action of glucocorticoids, which is the effect on gene expression through the interaction of GR with transcription factors that causes inhibition of NF-kβ and decreases the synthesis of pro-inflammatory molecules such as cytokines, adhesion molecules, and proteases.[8]

Binding affinity indicates the energy required to form bonds between ligands and the receptor. The lower the bond energy, the more stable the bond. A strong ligand binding can predict more significant ligand-receptor activity.[10] Even though the binding affinity is relatively high compared to hydrocortisone, clocortolone, triamcinolone, and beclomethasone dipropionate as control ligands, LTA still has potential in GR because it has the same binding site as GR compounds. The binding site is essential to determine where the drug's site of action is. An action target's binding site is a structural protein space that allows bonding between ligands and amino acid residues. The place of binding is also known as a binding pocket. A binding pocket is a concave protein surface with amino acid residues that function in the protein conformation mechanism.[10,20]

In silico studies on the anti-inflammatory properties of several ingredients have been carried out. A study on quercetin, the bioactive compound from onion *Allium cepa L* shows that quercetin compounds could bind the muscle-blind-like protein 1 receptor with binding affinity ranges from -5.7 to -6.5 kcal/mol. Dexamethasone as the control compound also binds to the same receptor with binding affinity ranges from -5.7 to -6.5 kcal/mol. This study shows that quercetin can be used as an anti-inflammatory agent.[15] *In silico* study on *Centella asiatica* as an anti-inflammatory agent indicates three active compounds of this herb that act as an inhibitor of IL-6, the pro-inflammatory cytokine. The three active compounds, asiatic acid, madecasid acid, and asiaticoside, have binding affinity to IL-6 with -9.8244, -9.7071, and -9.0171 kcal/mol, respectively.[21]

The skin sensitization potential has been conducted using the animal test. The ban on animal testing for cosmetics since 2013 has led to the development of non-

animal testing such as *in silico* methods. Skin sensitization prediction with *in silico* methods combines structural properties and computational methods. *Toxtree* is a rule-based structure-activity relationship (SAR) that identifies chemical structural alerts which indicate hapten binding.[22] This *in silico* study using *Toxtree* software means that the LTA of LAB is not irritative to the skin.

Lactic acid bacteria (LAB) formulations are now widely available as skin care products and are commonly used to prevent and treat skin diseases.[23] Some research on topical preparation of LAB had been conducted. A topical preparation of LAB was tested in healthy elderly and people with sensitive skin. In healthy elderly, the topical preparation of 0.5 g *Streptococcus thermophilus* twice daily for 7 days showed improved skin hydration, while the topical preparation of 10% extract *Bifidobacterium longum* twice daily for 2 months helped the irritated skin in the group of patients with sensitive skin. They were giving topical preparation of LAB to both groups that did not cause new skin problems. These studies demonstrate the safety and efficacy of topical preparation of LAB in healthy elderly and people with sensitive skin.[24]

A systematic review of a topical preparation of LAB in atopic dermatitis and seborrheic dermatitis has shown that this topical preparation can reduce erythema and scaling in both skin diseases. Application of 1,7 g/ 5 ml in 20 ml lotion of *Streptococcus thermophilus* twice daily in patients with atopic dermatitis provides improvement and enhancement of skin ceramides. Application of 0,3% *Lactobacillus johnsonii* twice daily for 21 days in patients with atopic dermatitis improves score of atopic dermatitis and reduces colonization of *Staphylococcus aureus*. Application of *Vitreoscilla filiformis* lotion for 4 weeks in patients with seborrhoeic dermatitis improves erythema, scaling, and pruritus. The effect of a topical preparation of LAB on the clinical improvement of these skin diseases is still unexplained.[24]

This study is a preliminary study to estimate the possibility of using LTA of LAB in inflammatory skin diseases through computer simulation. The limitation of the study is that this bioinformatics assessment has not been able to determine the actual efficacy and side effects in humans so that it can be continued with animal model research and followed up by clinical trial.

CONCLUSION

Based on bioinformatics assessment, we found that LTA of LAB has activity on GR and has the same binding site on GR as some glucocorticoid control compounds. The LTA toxicity analysis did not show irritative effects on the skin. Lipoteichoic acid (LTA), especially from LAB, needs to be studied further in animal models to explain the mechanism and in clinical trials to determine its efficacy in inflammatory skin diseases.

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ETHICAL CONSIDERATION

This research did not violate the rules of research ethics based on COPE and ICMJE protocols prior to the study being conducted.

CONFLICT OF INTEREST

The authors have no conflict of interest in this research.

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