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Brazilian Archives of Biology and Technology

Article - Human and Animal Health

Pharmacokinetic Profile of a Drug Repurposing Candidate for the Treatment of Cutaneous Leishmaniasis (in Silico)

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HIGHLIGHTS

- The theoretical prediction good solubility and permeability of chloroquine;

- Chloroquine presents proper physical chemical features for skin permeation;

- Chloroquine presented Ki= 4.86 µM against TR*Lb.*

Abstract: The calculated or experimental physical-chemical Properties are important for choosing substances that will be used in the treatment of several diseases by different administration routes. Chloroquine, for example, is a drug with several biological activities that has been constantly investigated as an alternative to drug repurposing in different diseases. With respect to leishmaniasis, there are few treatment options, which are invasive and have several adverse effects. Another point is the identification of important drug molecular targets, understand their functions, and thus discovering new therapeutic alternatives. Thus, this work aimed at: performing an in analysis of the pharmacokinetic profile of chloroquine as an option for the treatment of cutaneous leishmaniasis by dermal route; evaluating the interaction of the drug with the enzyme Trypanothione reductase, responsible for the parasite's redox balance. The melting point was obtained in the PUBCHEM database. Analysis showed that chloroquine presented a partition coefficient, molecular weight, and melting point within the established proper range of parameters. The skin permeability coefficient also presented a satisfactory value, as well as the values for total polar surface area, number of rotatable bonds, and sp3 Carbon Fraction. In molecular docking simulations, chloroquine showed interactions with the enzyme TR*Lb*, with a calculated Ki lower than that of the reference compound. This study reinforces the theoretical prediction and good solubility and permeability of chloroquine. The results justify further investigations on the inhibition of TR*Lb* as an important alternative for the treatment of leishmaniasis.

GRAPHICAL ABSTRACT

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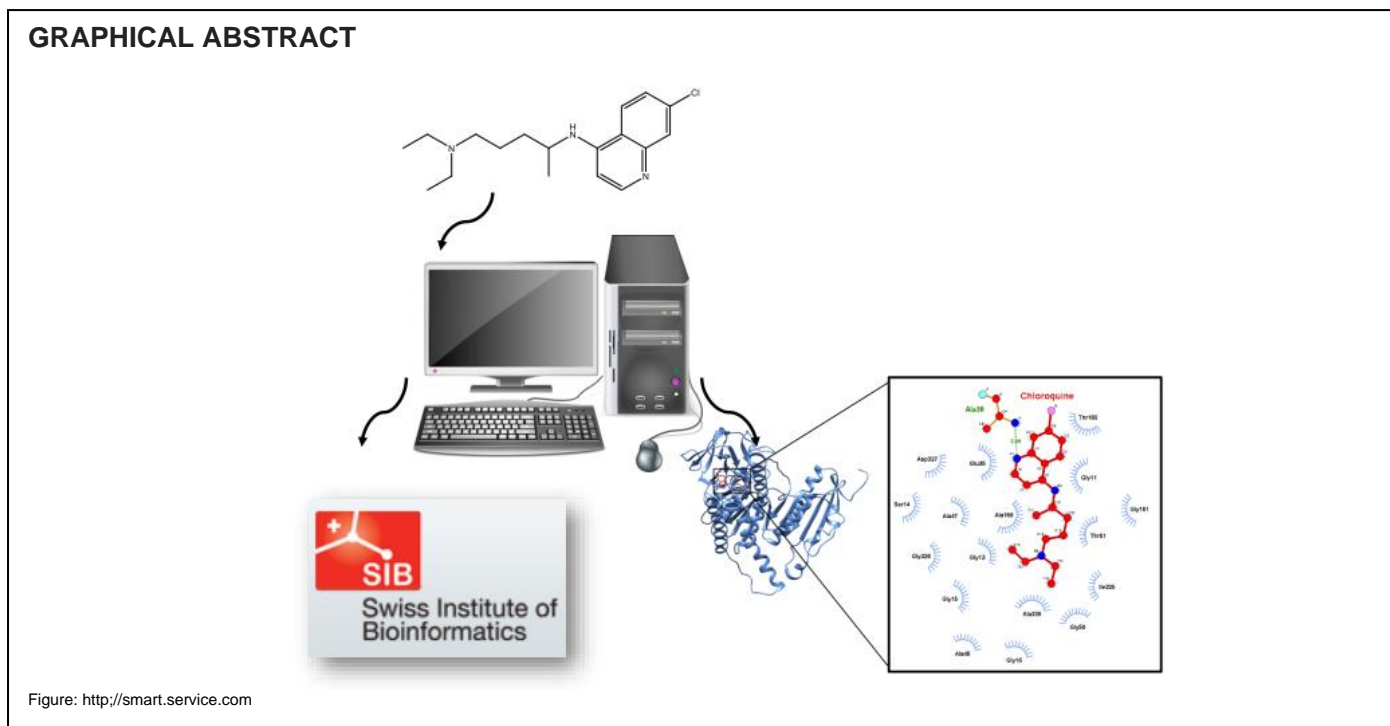
INTRODUCTION

Leishmaniasis is considered an infectious, non-contagious disease that belongs to the group of neglected tropical diseases (NTDs). About 12 to 14 million are infected, and 400 million of the world's population are at risk for this disease [1]. The genus *Leishmania* comprises approximately 30 different species, divided into two subgenera (*Viannia* and *Leishmania*) [2]. Furthermore, parasites are divided according to their clinical and developmental characteristics in the host, such as dermatropic and non-dermatropic [3]. This division in humans occurs as American Cutaneous Leishmaniasis (ACL), which causes manifestations in the mucosal, cutaneous and mucocutaneous forms of the disease (dermatotropic leishmaniasis) and visceral leishmaniasis (VL), which is also called kala-azar (non-dermatotropic) [2].

In Brazil, ACL is a public health problem, affecting mainly the low-income population, due to factors such as poor housing conditions, illiteracy, deficiency in the immune system, malnutrition associated with ACL, among others. Effective control of this pathology is achieved when selected public health approaches are combined and delivered locally [4,5].

The first-choice treatment is based on the use of pentavalent antimonial complexed to carbohydrates, in the form of sodium stibogluconate and meglumine antimoniate, and the second treatment of choice is Amphotericin B, a macrolide polyene antibiotic, acting on the ergosterol of the cell membrane. Both treatments have high toxicity and may harm the cardiac, hepatic, pancreatic, renal and musculoskeletal systems [6,7].

Topical administration of drugs is an alternative that has been investigated in recent years [8,9]. Transdermal drug delivery systems are already accepted as a way to achieve the release of drugs into the circulatory system via the skin [10,11]. In the case of ACL, the parasites are found in the dermal layer of the skin, internalized mainly by macrophages, as well as in Langerhans cells. Infected tissue can potentially be reached by topically applied substances. Therefore, transdermal administration of drugs is an interesting alternative for the treatment of the disease, considering that they are less invasive for users who have restrictions with respect to conventional treatments (intravenous or parenteral) [9,10,11,12,13]. In this sense, the theoretical or experimental physical-chemical properties are important for the choice of substances that will be applied by the dermal route [8].

Chloroquine is an aminoquinoline with antimalarial activity that can inhibit nucleic acid biosynthesis. It is a drug that has anti-inflammatory activity and chemosensitizing and radiosensitizing potential. It is effective in extra intestinal amebiasis and as an anti-inflammatory agent in the treatment of rheumatoid arthritis and lupus erythematosus. It is generally well tolerated, and may have mild side effects, such as

nausea, vomiting, toxic effects on the retina, which may occur with daily doses in the long term, and may affect the heart in acute toxicity [14,15].

Another point currently, is the identification of relevant molecular targets, understand their functions and then Search for new alternative treatments. For *Leishmania*, trypanothione reductase is an ideal target, since it is only found in flagellate protozoa such as *Leishmania* and *Trypanosoma*, besides exerting a vital role against oxidative stress, regenerating the main antioxidant present in these protozoa: trypanothione [16]. Another interesting fact, observed in Mutlu's work [17], is that in Leishmania species, reductions in peroxidase activity are directly caused by tryparedoxin peroxidase activity. Since the parasites are sensitive to oxidative stress, the enzymes involved in this pathway are attractive targets for potencial drugs.

Considering this information, this study aimed at evaluating the pharmacokinetic profile of chloroquine as an alternative to the topical treatment of cutaneous leishmaniasis and at evaluating the interaction of chloroquine with the enzyme Tripanothione reductase from *Leishmania braziliensis* (TR*Lb*). To this end, we opted for the use of *in o* techniques, which contribute to reducing the use of living beings in tests, faster data assembly and analysis, high replicability of the models (which in most cases can be made available free of charge and with lower costs), reduced need to assemble large experiments in laboratories, consumption of reagents and labor, in addition to equipment [18].

MATERIAL AND METHODS

Virtual screening

SwissAdme (http://www.swissadme.ch/) was used to evaluate the physical-chemical descriptors. The patterns predicted in the software are evaluated according to the model described by Daina, Michielin and Zoete [19].

Homology modeling

Homology docking was performed following the same guidelines as Silva and coauthors [16] to investigate the structural characteristics of the amino acids responsible for enzyme-ligand recognition and perform molecular docking, homology modeling of *Leishmania (V.) braziliensis* TR (GI: XP_001561849) was carried out.

The structure that served as a model was selected from the Protein Blast database (http://blast.ncbi.nlm.nih.gov) and the Protein Data Bank (PDB) (http://www.pdb.org) [20] having as a parameter the highest degree of similarity with TR*Lb*. Sequence alignments were performed using MODELLER v10.4 [21] and ClustalW [22] software. The construction of the model was performed in MODELLER v10.4. The protocol consists of generating a total of 1000 models, and the final model was selected based on the lowest Discrete Optimized Protein Energy (DOPE) scores calculated by the MODELLER software [23]. The general stereochemical quality of the final model for TR*Lb* was evaluated by the PROCHECK program [22]. Interactive visualization and comparative analysis of molecular structures were performed in the Swiss-PDB viewer [24] and UCSF Chimera [25].

Molecular docking simulations

In this approach, the program AutoDock 4.2 [26] was used through the interface AutoDock Tools. Once the three-dimensional models of TR*Lb* and the compounds were generated and validated, in order to visualize interactions, molecular docking was performed between the TR*Lb* target and chloroquine. The three-dimensional structure of chloroquine was obtained from the PubChem database and optimized using the UFF force field. Binding energy calculations were performed based on Lamarck's genetic algorithm [27]. The simulation grid was positioned in the active location of TR, centered at 31.267 Å, 58.680 Å and -8.853 Å on the x, y, z axes, respectively, with dimensions 92 Å × 93 Å × 94 Å and spacing of 0.375 Å between points from the grid. Interaction analyzes were acquired in Ligplot+ v.2.2.8 [28] and visualization in UCSF Chimera [20].

RESULTS AND DISCUSSION

Theoretical or experimental physical-chemical properties are important for choosing substances that will be used by the dermal route. Considering the natural selectivity of the skin, for transdermal application of molecules, it is important that they present certain requirements such as: partition coefficient (logP) between 1.0 and 4.0 to cross the stratum corneum and reach the bloodstream; molecular weight ≤ 500 g/mol; and the melting point ≤ 200 ºC are also properties that allow for determining the solubility of the drug

in the stratum corneum [29-31]. The Kp (skin permeability coefficient) was also evaluated: the more negative the log Kp (with Kp in cm/s), the less permeant to the skin is the molecule, according to Table 1.

Table 1. Physical-chemical Properties of Chloroquine

	MM (g/mol)	LogP o/w	Kp (cm/s)	TPSA (Å^2)	NR	Fraction Csp3	Pf (ºC)
Chloroquine	319.87	3.95	-4.96	28.16	8	0,50	87 – 89,5

MM: molar mass; LogP o/w: logarithm of the oil-water partition coefficient; Kp: skin permeability coefficient; TPSA: the polar surface area occupied by nitrogen and oxygen atoms bonded or not to hydrogen; NR: Number of rotatable bonds; Csp3 fraction: sp3 carbon fraction; Mp: melting point. Data were obtained using the SwissAdme software. The melting point was obtained from the PubChem database.

There are other descriptors that can help to better understand the limiting factors of the permeation process, such as the polar surface area (TPSA, Topological Polar Surface Area, ≤ 140 Å), the number of rotational bonds (NR, Number Rotatable Bonds ≤ 10 [32], to facilitate drug permeability) and the sp3 carbon fraction (Fsp3) [33].

In the bioavailability radar (Figure 1), it is possible to verify six physical-chemical properties taken into account for bioavailability: lipophilicity, molecular size, polarity, solubility, flexibility and saturation.
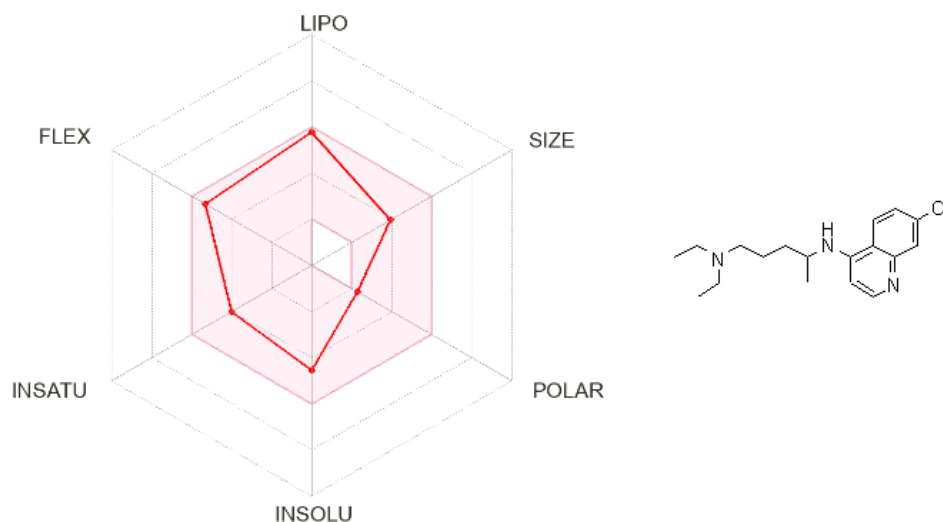

Figure 1. Chloroquine bioavailability radar – SwisAdme. Chloroquine. Image generated with the software ChemDraw Professional Version: 18.1. The pink area represents the ideal range for each property: LIPO (lipophilicity)= XLOGP3 between -0.7 and +5.0; SIZE (molecule size) = between 150 and 500 g/mol; POLAR (polarity)= TPSA between 20 and 140 Å2; INSOLU (insolubility)= LOG S <6; INSATU (saturation)= sp3 carbon fraction ≥0.25; FLEX (flexibility) ≤9 rotatable groups.

In this analysis, the gold standard of bioavailability is achieved when all parameters are within the pink area of the graph. The results reinforce the prediction of good solubility and permeability of chloroquine.

According to Veber and coauthor [32] fewer rotating bonds (≤ 10) are ideal for conformational stability of molecules and passage through membranes.

The carbon fraction can be described as the ratio of sp3-hybridized carbons to the number of total carbons in the compound. According to Lovering, Bikker, and Humblet [34] drugs that have an sp3 carbon fraction (Fsp3) equal to or greater than 0.47 have better solubility due to the solvation capacity of water.

The increase in saturation makes the structure of the compound more complex, allowing different chemical states to be explored. This effect may allow greater complementarity with molecular targets that are more complex and inaccessible to flat molecules, for example. Thus, increasing complexity can increase the potency and/or specificity of a drug candidate for the active site without significantly increasing the molecular weight [34].

Chloroquine is a substance that has a hydrophilic moiety (water-soluble), which is an important property for treatments by the dermal route, considering that the reduced TPSA shows better correlation with an increase in permeation rate than the partition coefficient (logP), taking into consideration that the substance needs to be hydrophilic enough to feasibly cross the epidermis, once the desolvation of polar

groups is necessary for permeation. In this sense, lipophilicity is needed to conduct the solutes to the interfacial region of the membrane. Thus, substances with a balance between water hydrophilicity and lipophylicity are properly absorbed by the skin [32,35].

Molecular docking

The structure of Trypanothione Reductase from *Leishmania infantum* (PDB ID: 2JK6) was selected as a model, having >80% similarity with the TR sequence from *Leishmania* (*V.*) *braziliensis*. The BLAST search revealed many possible models of high-level similarity to the target sequence. The percentage of residues lying in the favored regions of a Ramachandran plot [31] is one of the best guides for checking the stereochemical quality of a protein model based on the assumption that the model should have more than 90% of residues in the allowed regions [23].

Analysis of the Ramachandran plot for the modeled structure resulted in more than 92% of the amino acids being in favorable regions and none in forbidden regions [36]. The quality of the model was also evaluated, comparing the predicted structure with the structure of the model through the evaluation of overlap and root mean square deviation (RMSD) of the atoms. The RMSD tracking of αC atoms (alpha carbon) across all structures and homology models is less than 1.00 Å. Thus, the support for the generated model is reasonably good and quite similar to the original model. The results of the best dimeric interaction between *Leishmania* (V.) *braziliensis* TR and the ligands are based on the binding energy of the receptor-ligand complex.

Below are represented the structures modeled for chloroquine and TR*Lb*, as well as the main aminoacid residues involved in the interaction via hydrogen bonds and hydrophobic interactions with the aminoacids (Figure 2).
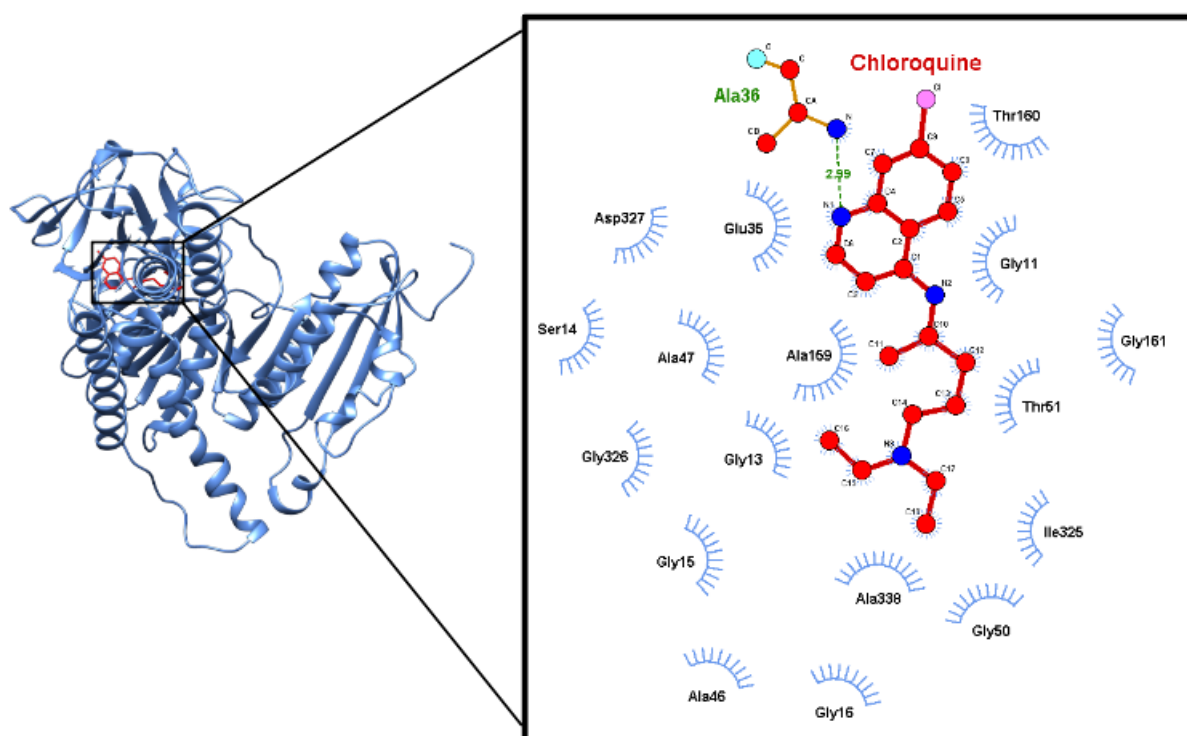

Figure 2. Modeling of Trypanothione reductase from *Leishmania* (*V.*) *braziliensis* and docking of chloroquine. Images generated by UCSF Chimera and Ligplot+ software v.1.4.5.

Binding analysis of TRLb with the ligand identified specific amino acid residues (Asp327, Ser14, Gly15, Ala46, Ala47, Gly50, Ile325, Glu35, Ala159, Gly13, Ala338, Thr51, Gly11 and Thr160) within the hydrophobic binding pocket of TRLb to play an important role in binding affinity. Chloroquine was shown to form a hydrogen bond with Ala36 (2.99 Å) as shown in Figure 2.

The docking conformation of chloroquine showed a predicted binding free energy of -7.25 kcal mol^{-1} to Trypanothione reductase with a theoretical inhibition constant (Ki) of 4.86 µM at the temperature of 298.15 K.

The first reports of molecular modelling studies in the Search for compounds that block trypanothione reductase were shown by Benson and coauthors [37]. In the study, the authors demonstrated that some antidepressive compounds act by inhibiting. One of these compounds, clomipramine, was shown to obtain a Ki of 6.6 μM, not inhibiting human glutathione reductase at the maximum concentration (1 mM). Our study demonstrated that chloroquine has a calculated Ki better than that of clomipramine, justifying further assays (enzyme *in vitro* studies) to confirm the thesis.

CONCLUSION

Through the analyzes carried out in the SwissAdme software, it is possible to predict that the drug chloroquine has adequate physicochemical characteristics for skin permeation, as well as a balance between water solubility and liposolubility, a favorable feature for a substance to be absorbed by the skin. Our study demonstrated that chloroquine exhibited binding interactions with the TR*Lb* enzyme, with a theoretical Ki better than that of the reference inhibitor (clomipramine) reported in the literature, which justifies further investigations such as enzymatic assays (*in vitro*) of the substance with the molecular target.

Conflict of interest: the authors declare that there are no financial interests or personal relationships that could have influenced the work reported in this article.

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