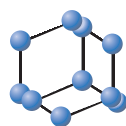
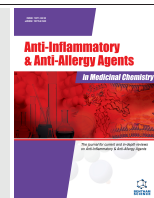


RESEARCH ARTICLE

BENTHAM
SCIENCE

In silico and *in vitro* Study of the Inhibitory Effect of Anti-inflammatory Drug Betamethasone on Two Lipases



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Abstract: Background: For the first time, the anti-inflammatory drug betamethasone is investigated for its inhibitory activity against lipase.

Objective: This work aims to demonstrate the *in vitro* and *in silico* inhibitory effect of the anti-inflammatory drug betamethasone on the enzymatic activity of two lipases.

Methods: *In vitro* study using p-nitrophenyllaurate as lipase substrate is used to determine inhibition potency. Molecular Docking is performed using the Autodock Vina for drug molecule and two enzymes *Candida rugosa* lipase and human pancreatic lipase.

Results: Betamethasone represents a moderate inhibition effect with a value of IC₅₀ of 0.36±0.01 mg/ml. Molecular docking allowed us to understand inhibitory – enzyme interactions and to confirm *in vitro* obtained results.

Conclusion: These experiments showed that betamethasone can be used in the treatment of diseases related to lipase activity.

Keywords: Anti-inflammatory drugs, autodock vina, betamethasone, *Candida rugosa*, human pancreatic lipase, diseases related to lipase, inhibition, lipase, molecular docking.

1. INTRODUCTION

Lipase is one of the essential enzymes of the digestive process. It is widespread in nature and has an important physiological role in the metabolism of fats, that is, the hydrolysis of triglycerides to diglycerides, monoglycerides, fatty acids and glycerol [1, 2]. The excess lipids cause significant health problems such as hyperlipidemia and obesity.

Human obesity is a complex chronic disease, characterized by an abnormal or excessive accumulation of body fat that can affect health. Obesity

results from an imbalance between energy intake and energy expenditure [3, 4]. Obesity is a factor that can intervene in many diseases: coronary artery disease, cardiovascular diseases, diabetes, and certain cancers [1, 3, 5].

In the cases where the diet is not enough to fight these diseases, pharmacological treatments are then proposed. Current research is increasingly interested in products that induce a more general inhibition of lipolytic activity [6, 7].

Lipase inhibitors can be found naturally in plants and can be produced as drugs. Currently, the best-known drug used for the treatment of obesity is Orlistat. It is a potent inhibitor of human pancreatic lipase [8]. This medication has side effects such as; diarrhea, bloating, oily spots, flatu-

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lence, dyspepsia and faecal incontinence [9]. That is why more research studies of new lipase inhibitors are necessary.

Anti-inflammatory medicines form a vast family of compounds apparently very heterogeneous since they consist of substances with very diverse chemical structures. Anti-inflammatory drugs are among the most widely prescribed medications worldwide, they represent treatment of various inflammatory diseases such as arthritis, rheumatism, relieve the pains of daily life [10].

Betamethasone is classified as a steroid drug, it is used to treat many diseases such as: rheumatoid arthritis, dermatitis and asthma. The anti-inflammatory effects of betamethasone start in about two hours and last for seven days.

In this study, for the first time, inhibitory activity against *Candida rugosa* lipase of some anti-inflammatory drugs was investigated *in vitro*. Bethametasone is the only one that represents a moderate inhibition effect while the other drugs represent a very low inhibitory effect, that's why we are interested in betamethasone.

In addition, we used molecular docking to expect different interactions between the potent drug and two enzymes; *Candida rugosa* lipase and human pancreatic lipase.

2. EXPERIMENTAL

2.1. Drugs and Chemicals

Candida rugosa lipase, p-nitrophenyl laurate, and all other reagents were purchased from Sigma-Aldrich. All other chemicals and solvents were purchased from Sigma- Aldrich. Drugs were purchased from a Pharmacy shop.

2.2. Drug Preparation

Serial dilutions of the liquid drug betamethasone were prepared in phosphate saline buffer with pH 7. The 2D structure is presented in Fig. (1).

2.3. *C. rugosa* Lipase Assay

The method used for *C. rugosa* lipase was previously described and performed [5, 11, 12] with some modification. The non-colored substrate p-NPL was dissolved at 1 mM in 2-propanol and

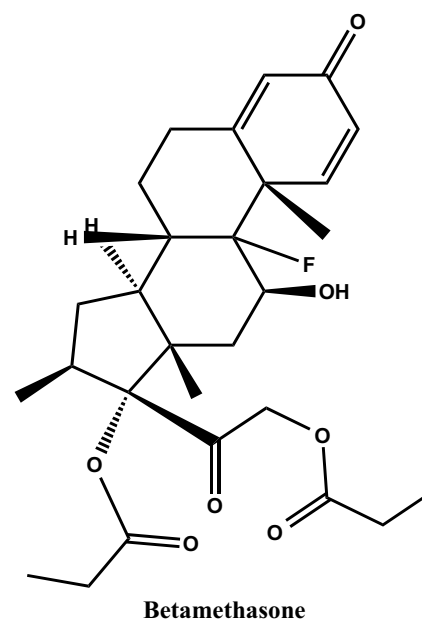


Fig. (1). 2D chemical structure of betamethasone.

mixed by vortex for 3 min. A 1:10 (v/v) dilution in phosphate saline buffer with pH 7 was prepared with gentle agitation. For determining lipase inhibitory activity, 20 μ l of serial dilutions of drug were pre-incubated with 20 μ l of enzyme solution (0.5 mg/ml) for 15 min at 37°C. Then, 180 μ l of substrate p-NPL solution was added and the reaction mixture was incubated for 15 additional minutes at 37°C. The absorbance of p-nitrophenol (the yellow-colored product of the reaction) was immediately measured in microplate reader Biotek ELX 800 UV at 405 nm. In the positive control, the drug was replaced with the same volume of buffer. Negative controls were also applied to check the activity with and without inhibitor. All tests were performed in tetraplicate. The lipase inhibition (I%) was calculated according the following formula:

$$I\% = \left(1 - \left(\frac{A_{drug}}{A_{control}} \right) \right) \times 100$$

where, A_{drug} : is the activity with drug (inhibitor), $A_{control}$: the activity without inhibitor [3]. The concentrations yielding a lipase inhibition of 50% (IC₅₀) were calculated from the inhibition vs. drug concentration curves by regression analysis.

2.4. Molecular Docking

The drug betamethasone (celestene) was obtained from PubChem database, it was assembled

with Discovery Studio visualizer v2016. The 3D structure of *C. rugosa* lipase (PDB ID: 1LPP) and human pancreatic lipase (PDB: 1LPB) was obtained from protein data bank (PDB).

For docking studies, the initial proteins were prepared by removing all the water molecules, heteroatoms, any co-crystallized solvent and ligands. It is well-known that the PDB files have missing hydrogens. Hence, the polar hydrogens and partial charges were added to the structure using Autodock tools (ADT) (version 1.5.6) [13]. Docking calculations were performed with AutoDock Vina program [14] in an eight CPU station. Because it uses rectangular boxes for the binding site, the box center was defined and the docking box was displayed using ADT. Docking was specific and rigid for LCR with a grid box of 22 x 20 x 24 and grid points were separated by one Å, it was positioned at the middle of the protein (x = 65.462; y = 52.896; z = -10.469). The docking was specific and rigid for HPL with a grid box of 10 x 22 x 18 and grid points separated by 1 Å, it was positioned at the middle of the protein (x = 9.739; y = 26.117; z = 51.06). Default parameters were used except the number of output conformations was set to one. The number of runs was 50 in order to get 50 solutions. The searching seed was random. The preferred conformations were the ones of lowest binding energy within the active site. The ratios of repetitions of the best solutions were determined. Finally, the docking results generated were directly loaded into Discovery Studio visualizer, v 2016 [11, 15].

3. RESULTS AND DISCUSSION

We evaluated the inhibitory effect of our drugs on the activity of this enzyme. Among six anti-inflammatory drugs, only one drug showed good inhibition which we proceeded for IC₅₀ determination.

The IC₅₀ value was determined for betamethasone that gave a percent inhibition more than 50% for the concentration of 1 mg/ml. The results showed IC₅₀ of betamethasone = 0.36 ± 0.01. Comparing this result with those published in [5], we confirm that our drug betamethasone is twice more potent than Folic Acid and febuxostat with IC₅₀ values = 0.64 and 0.66 mg/ml. Compared to orlistat (IC₅₀ = 0.06 mg/ml), our studied drug is six times less potent than the common drug used as lipase inhibitor.

In this approach, a docking simulation of the studied drug was carried out by targeting the active site of the crystalline structure of the two lipases (PDB ID: 1LPP and 1LPA) in order to define the nature of the interactions between the betamethasone and the enzymes using AutoDock Vina program [14]. We selected the best docking pose for each complex enzyme-inhibitor, based on the number of repetitions and the type of interaction existing in 50 solutions.

Through this study, we will discuss the type of interactions of inhibitory drug Betamethasone with *Candida rugosa* lipase (Fig. 2) and human pancreatic lipase (Fig. 3). The results of Docking (Table 1) related to the number of obtained solutions show

Table 1. The results of interactions between betamethasone and both lipases.

Drugs	Rate Repeat %	Free Binding Energy (kcal mol ⁻¹)	Closest residues	Hydrophobic Interactions	Hydrogen Bonds	Length (Å)
<i>Candida rugosa</i> Lipase						
Betamethasone	100	-7.4	Val127 Pro65	alkyl -alkyl alkyl -alkyl	/	/
HumanPancreatic Lipase						
Betamethasone	100	-8.2	Phe77, Ile78, Tyr114, Phe215, Ala259	Pi-alkyl Alkyl- alkyl	/	/

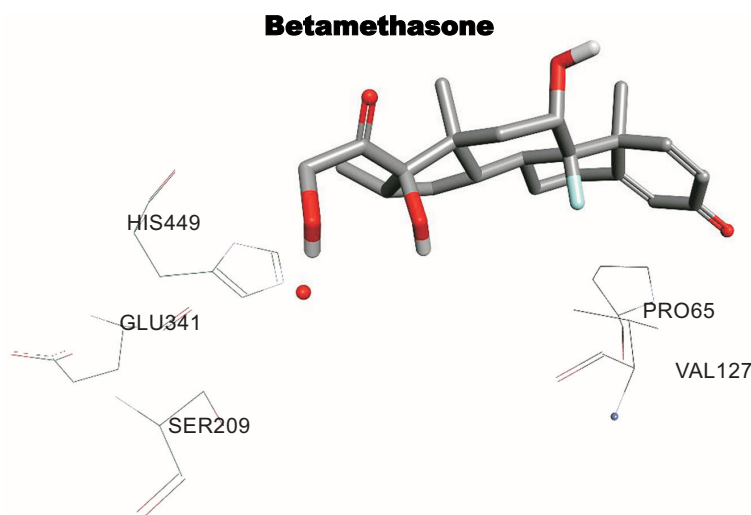


Fig. (2). Best Binding mode of Betamethasone at the active site of *Candida rugosa* lipase. Betamethasone is illustrated in gray. Ligand is rendered as sticks. Different subsites of the active site were colored: catalytic triad (CT) in green, binding site amino acids are colored in black. (A higher resolution / colour version of this figure is available in the electronic copy of the article).

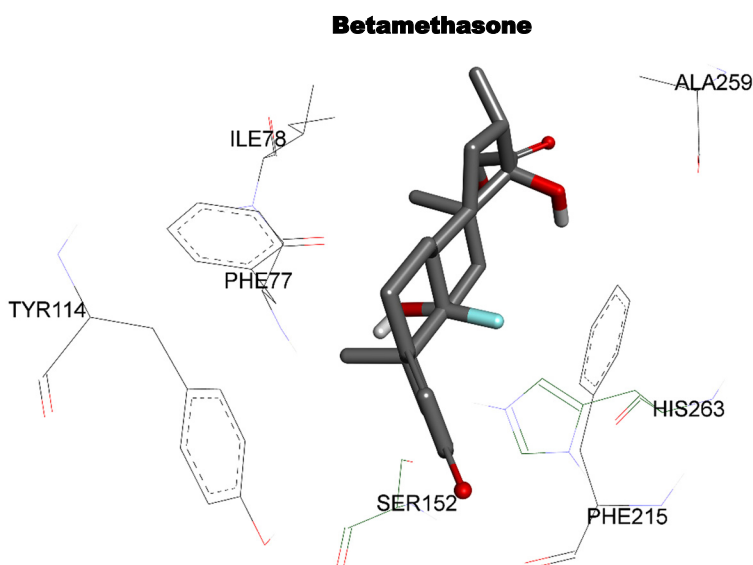


Fig. (3). Best Binding mode of Betamethasone at the active site of *human pancreatic lipase*. Betamethasone is illustrated in gray. Ligand is rendered as sticks. Different subsites of the active site were colored: catalytic triad (CT) in green, binding site amino acids are colored in black. (A higher resolution / colour version of this figure is available in the electronic copy of the article).

that betamethasone have both lipases inhibition activity, which confirms the experimental results.

We obtained a high repetition rate equal to 100% of the same conformation, and an affinity energy of -7.4 Kcal / mol. The *in vitro* study of Betamethasone shows that it has an important inhibitory activity for *Candida rugosa* lipase. The same result is shown by the *in silico* study where it interacts with two amino acids of cavity site

Val127 and Pro65, the types of the involved interactions are alkyl-alkyl in the same site with the Betamethasone cycles. Hence, Betamethasone is very well inserted into the active site of the LCR enzyme. No hydrogen bonds are recorded in this position (Table 1).

For HPL, betamethasone is capable of forming hydrophobic interactions (two Pi-alkyl), (three alkyl-alkyl), (two Pi-alkyl) and alkyl-alkyl with

amino acids of the active site of HPL: Phe77, Ile78, Tyr114, Phe215 and Ala259, respectively, with a repetition rate equal to 100% and an affinity energy of -8.2 Kcal / mol. This inhibitor does not form any hydrogen bonds. According to this result, betamethasone is a good inhibitor for HPL. Comparing this result with those cited [5], colchicine, Folic acid and Febuxostat interact in different manners with different amino acids of the catalytic cavity. The difference is presented mainly in the binding mode of the studied drug and the others.

CONCLUSION

This study was restricted for the first time to the screening of anti-inflammatory drug as potent inhibitor of lipases, we have found that betamethasone is the best inhibitor or both lipases but not more effective than orlistat. Further needed studies should be achieved for other drugs to detect their potentialities.

LIST OF ABBREVIATIONS

HPL = Human Pancreatic Lipase

LCR = Lipase of *Candida rugosa*

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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