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Determination of Pharmacokinetic and Toxicological Parameters of Some Commonly Used Statin Group Drugs

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Abstract: Statins work as inhibitors of the HMG-CoA reductase enzyme and are the most commonly prescribed cholesterol-lowering drug group for people with cardiovascular disease or risk. This study aimed to determine the pharmacokinetic parameters and toxicities of conventional and new-generation cholesterol drugs such as atorvastatin, fluvastatin, lovastatin, pravastatin, simvastatin, rosuvastatin and pitavastatin. Absorption, distribution, metabolism, and excretion (ADME) parameters and toxicity predictions of drugs were made using in silico modeling, which gives faster results than animal experiments and does not involve any laboratory costs. In calculations made using structural similarity, compounds' pharmacokinetic properties and toxicity predictions are obtained based on previously known structure-activity data. The study results showed that the toxicity classifications of the drugs were 5 (LD: 5000 mg/kg) for atorvastatin and 6 (LD: 8939 mg/kg) for pravastatin, respectively. The toxicity classes were found to be 4 for all the other statin group drugs. The results showed that pravastatin had the lowest toxicity among investigated cholesterol drugs, while pitavastatin and fluvastatin had the highest toxic effects. Accordingly, it is recommended that the consequences of using pravastatin and atorvastatin, cholesterol drugs with lower toxicity classes, should be investigated more seriously in terms of minimum toxic substance intake for patient groups requiring high doses of medication.

Yaygın Olarak Kullanılan Bazı Statin Grubu İlaçların Farmakokinetik ve Toksikolojik Parametrelerinin Belirlenmesi

Anahtar Kelimeler
Statin grubu ilaçlar,
Moleküler Modelleme,
In silico incelemeler

Öz: Statinler, HMG-CoA redüktaz enziminin inhibitörleri olarak çalışır ve kardiyovasküler hastalığı veya riski olan kişiler için en sık reçete edilen kolesterol düşürücü ilaç grubudur. Bu çalışmada atorvastatin, fluvastatin, lovastatin, pravastatin, simvastatin, rosuvastatin ve pitavastatin gibi geleneksel ve yeni nesil kolesterol ilaçlarının farmakokinetik parametrelerinin ve toksisitelerinin belirlenmesi amaçlandı. İlaçların emilim, dağılım, metabolizma ve atılım (ADME) parametreleri ve toksisite tahminleri, hayvan deneylerine göre daha hızlı sonuç veren ve herhangi bir laboratuvar maliyeti gerektirmeyen in silico modelleme ile yapılmıştır. Yapısal benzerlik kullanılarak yapılan hesaplamalarda, bileşiklerin farmakokinetik özellikleri ve toksisite öngörüleri, önceden bilinen yapı-aktivite verilerine dayanılarak elde edilmiştir. Çalışma sonuçları, ilaçların toksisite sınıflandırmalarının atorvastatin için sırasıyla 5 (LD: 5000 mg/kg) ve pravastatin için 6 (LD: 8939 mg/kg) olduğunu gösterdi. Diğer tüm statin grubu ilaçlar için toksisite sınıfı 4 olarak belirlendi. Sonuçlar, araştırılan kolesterol ilaçları arasında pravastatinin en düşük toksisiteye sahip olduğunu, pitavastatin ve fluvastatinin ise en yüksek toksik etkilere sahip olduğunu gösterdi. Buna göre, düşük toksisite sınıfına sahip kolesterol ilaçları olan pravastatin ve atorvastatinin yüksek doz ilaç gerektiren hasta gruplarında kullanımının minimum toksik madde alımı açısından sonuçlarının daha ciddi şekilde araştırılması önerilmektedir.

1. INTRODUCTION

Cardiovascular diseases (CVDs) refer to pathologies occurring in the heart and vascular pathways and are among the important causes of death, especially in underdeveloped and developing countries [1]. One of the most critical factors of cardiovascular diseases is the increase in the level of high low-density lipoprotein (LDL) in blood, popularly referred to as bad cholesterol. LDL can cause atherosclerosis and, as a result, the development of coronary heart disease. Statins are treatment agents that control LDL cholesterol by inhibiting the HMG-CoA reductase enzyme [2, 3]. Due to these features, they provide primary protection for people at high risk of cardiovascular disease. At the same time, they are also used in secondary protection in patients who have had cardiovascular disease [4].

Nowadays, the forms of statin commonly used to keep the blood-cholesterol level in a certain balance can be listed as atorvastatin, fluvastatin, lovastatin, pravastatin, simvastatin, rosuvastatin and pitavastatin [5]. Although each of these forms of statins has different pharmacokinetic properties and activities, they also have serious side effects, such as hepatoxicity and nephrotoxicity. However, it is well known that there is no drug without side effects. In clinical use, the physician evaluates the patient's general condition, and the most appropriate drug (most effective and with the lowest side effects) is always recommended.

The most important process after taking a drug into the body is that the drug causes the least harm to the body (which can be expressed as minimum toxicity) and provides maximum benefit. Absorption, distribution, metabolism, and excretion (ADME) processes are critical in ensuring maximum benefit after the dose with minimum toxicity is given to the patient. In this respect, obtaining ADME data in pharmacokinetic processes can provide useful information at the preclinical stage of drug development. In this respect, in silico ADME calculations can show the potential of a new molecule to be used as a drug.

In this study, ADME, drug-likeness, and toxicity predictions of some important statin group drugs were performed in silico. Which drug has what kind of toxicity and LD50 values have been determined. Blood-brain barrier penetration, Caco2 and MDCK cell permeability, and HIA parameters were also obtained. From the results obtained, toxicity situations that may arise as a result of the use of drugs were interpreted.

2. MATERIAL AND METHOD

The 2D molecular structures of the statin group drugs used in this study were drawn using the MarvinSketch program [6]. In the part of in silico biological activity calculations, the ADME and drug-likeness parameters were obtained using the PreADMET [7] website. Toxicity predictions for each drug were performed using the ProTox-II [8] suite.

3. RESULTS

3.1. Molecular Structures and Mechanism of Action of Statin Group Drugs

Statins are generally divided into two groups: those produced by fermentation (type I statins) and those produced synthetically (type II statins). Lovastatin, simvastatin and pravastatin are the first three natural statins produced for clinical use [9]. Lovastatin and pravastatin were isolated from broths as a result of fermentation. Simvastatin is a semi-synthetic statin derived from lovastatin produced by fermentation. Fluvastatin, atorvastatin, pitavastatin and rosuvastatin are synthetically obtained statins. The molecular structures of the drugs are given in Figure 1.

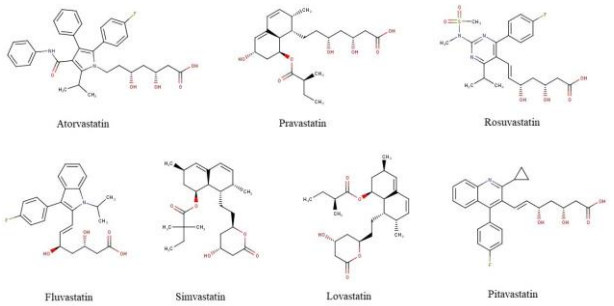

Figure 1. 2D molecular structures of statin group drugs

As can be seen from Figure 1, statins are structurally large molecules and contain many rings and chains in their structures. While lovastatin and simvastatin lack the polar 'head' group in the generalized system, these compounds are pro-drugs [10]. Their respective lactone rings are hydrolyzed in vivo to produce the corresponding hydroxyacid form. Essentially, statins bind to active moieties in the hydrophobic binding sites of the HMG-CoA reductase enzyme, which is actively used for cholesterol synthesis and competitively inhibits the functioning of this enzyme. This interaction is stronger than natural substrate binding. HMG-CoA reductase enzyme is a protein located in the HMG-CoA reductase pathway and used for cholesterol synthesis. In addition to inhibiting cholesterol synthesis, statins are also effective in regulating intracellular cholesterol levels. By reducing intracellular cholesterol levels, they increase LDL receptor synthesis in liver cells and thus accelerate the removal of LDL from the blood [11].

3.2. ADME Parameters of Statins

In medical and biological applications, there are some critical parameters in applying a molecule with therapeutic effects to the patient as a drug. These parameters are absorption, distribution, metabolism, and excretion (ADME) processes, and the optimal benefit of the drug to the patient is directly related to these factors [12]. In this respect, obtaining ADME data in pharmacokinetic processes can provide useful information at the preclinical stage of drug development. These parameters can also provide helpful information in evaluating drug effectiveness after administration. One of the ADME parameters that can be calculated in silico is Blood-brain barrier penetration (BBB), which predicts

whether compounds pass across the blood-brain barrier. The central nervous system (CNS) active compounds are more than 1.0 (Cbrain/Cblood). The second one is Caco2 cell permeability. Caco-2 cells are derived from human colon adenocarcinoma and possess multiple drug transport pathways through the intestinal epithelium. Another one is Madin-Darby canine kidney (MDCK) cells permability. It is a model mammalian cell line used in biomedical research. Middle permeability is 4~70, low permeability is less than 4, and high permeability is more than 70 nm/sec.

HIA-Human Intestinal Absorption predicts the percentage of human intestinal absorption (% HIA). Human intestinal absorption data are the sum of bioavailability and absorption evaluated from the excretion ratio or cumulative excretion in urine, bile, and feces. Well-absorbed compounds are 70~100 %.

The calculated ADME parameters for the statins examined in this study are given in Table 1.

Table 1. In silico predicted ADME parameters of the statins

Statin	**Atorv**	**Fluv**	**Lov**	**Prav**
BBB	0.673	0.174	0.664	0.067
Caco2	21.709	17.489	27.516	18.943
HIA	94.651	95.075	96.566	77.691
MDCK	0.044	0.045	0.056	0.084
Pure water solubility (mg/L)	0.115	9.045	5.458	405.165
Skin Permeability	-2.419	-2.452	-2.692	-3.080
Plasma Protein Binding	90.348	91.606	100.00	85.811
Statin	**Simv**	**Rosuv**	**Pitav**	
BBB	1.147	0.106	0.054	
Caco2	29.741	0.505	19.490	
HIA	96.564	90.483	95.163	
MDCK	0.047	0.105	0.055	
Pure water solubility (mg/L)	2.134	9.440	0.660	
Skin Permeability	-2.159	-2.430	-2.742	
Plasma Protein Binding	100.00	96.199	96.269	

As can be seen from the table, Simvastatin is the only statin group drug with BBB penetration above 1. It is also the cholesterol drug with the highest Caco2 value, while Rosuvastatin has the lowest value. When the calculated % HIA parameters are examined, it is seen that all of them are above 70%. However, while Pravastatin has the lowest HIA value, the absorption of other drugs is over 90%. When MDCK cell permeability values, another important ADME parameter, are examined for each drug, it is seen that they are well below 1 for all drugs, that is, all of the drugs have low permeability. One of the critical factors in the absorption and distribution of a drug within the body is water solubility. Pravastatin has the highest solubility in water, with 405.165 mg per liter, while surprisingly, the solubility for other statin group drugs is below 10 mg/L. Plasma protein binding (PPB) refers to the degree to which medications attach to blood proteins within the plasma [13]. A drug's efficacy is related to the degree to which it binds. The less bound of a drug mean the more efficiently it can diffuse through cell membranes. In this respect, Pravastatin may have higher medical efficacy than other statins, with the lowest PPB value as well as the highest water solubility.

3.3. Toxicity of the Compounds

Toxicity is the degree to which a chemical or a mixture causes harm to the organism [14]. Toxicity can refer to the damage of the toxic substance on the whole organism, or it can refer to the effect on a group of cells or an organ in the organism. One of the most important features of toxicity is the dose dependence of the effects of the toxic substance. Even substances with very high toxicity (such as snake venom) may have no toxic effects when taken in very small doses. Additionally, toxicity is a species-specific concept. Substances that show high toxicity for some species are not effective on some species.

The toxicity effects on the target tissue or cell can be measured in various units. Lethal dose (LD50) values generally determine toxicity definitions. It is a measure of toxicity, usually at the population level, that relates LD50 estimates to the probabilities of an outcome for a given individual in a population of individuals who respond differently to the same dose of toxicant [15].

The in silico predicted LD50 values of the statins are given in Table 2.

Table 2. In silico predicted ADME parameters of the statins

Statin	**Predicted LD50 mg/kg**	**Toxicity**	**Predict. Toxicity Class**	**Molecular Structure**
Atorv.	5000	Hepatotoxic	5	$C_{33}H_{35}FN_2O_5$
Fluv.	416	Hepatotoxic	4	$C_{24}H_{26}FNO_4$
Lov.	1000	Carcinogenic Immunotoxic	4	$C_{24}H_{36}O_5$
Prav.	8939	Carcinogenic Immunotoxic	6	$C_{23}H_{36}O_7$
Simv.	1000	Carcinogenic Immunotoxic	4	$C_{25}H_{38}O_5$
Rosuv.	464	Hepatotoxic Immunotoxic	4	$C_{22}H_{28}FN_3O_6S$
Pitav.	416	Hepatotoxic	4	$C_{25}H_{24}FNO_4$

Toxicity classes and LD50 (mg/kg) values are defined as follows: Class I: fatal if swallowed (LD50≤5) Class II: fatal if swallowed (5<LD50≤50) Class III: toxic if swallowed (50<LD50≤300) Class IV: harmful if swallowed (300<LD50≤2000) Class V: may be harmful if swallowed (2000<LD50≤5000) Class VI: non-toxic (LD50>5000) [8]. The LD50 values of the statins were calculated as 5000 mg/kg, 416 mg/kg, 1000 mg/kg, 8939 mg/kg, 1000 mg/kg, 464 mg/kg and 416 mg/kg, respectively. Toxicity classes were obtained as 6 for Pravastatin, 5 for Atorvastatin and 4 for all other drugs. The results show that Pravastatin has the lowest toxicity among the drugs and the predicted result was obtained as non-toxic. However, if taken in very high doses, it may have carcinogenic and immunotoxic effects. The ones showing the highest toxicity are Fluvastatin and Pitavastatin. Both drugs may exhibit hepatoxic effects.

4. DISCUSSION AND CONCLUSION

In this study, toxicity and ADME predictions of some important statin group drugs were performed in silico. The study results show Pravastatin has the lowest toxicity among investigated cholesterol drugs. Although it is the least toxic statin, also has the lowest Human Intestinal Absorption value. The second drug with the lowest toxicity was estimated to be atorvastatin. However, atorvastatin is most preferred in treatment due to its lower

toxic effect and broader spectrum lipid carrier protein level control effect. Accordingly, it is recommended that the consequences of using pravastatin and atorvastatin, cholesterol drugs with lower toxicity classes, should be investigated more seriously in terms of minimum toxic substance intake for patient groups requiring high doses of medication. The structure-activity-related computational studies can provide useful preliminary information to researchers..

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