INSIGHTS INTO THE INTERACTION BETWEEN STATINS AND MONOCARBOXYLATE TRANSPORTER 1: A MOLECULAR DOCKING APPROACH

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by

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LIST OF ABBREVIATIONS

SRM	Statin-Related Myopathy
SAMS	Statin-Associated Muscle Symptoms
MCT	Monocarboxylate Transporters
LDH	Lactate Dehydrogenase
PBS	Phosphate-Buffer Saline
DMEM	Dulbecco's modified Eagle's Medium
IC50	Inhibitory Concentration 50%
ETC	Electron Transport Chain
rt-qPCR	Quantitative Reverse Transcription Polymerase Chain Reaction
ROS	Reactive Oxygen Species
TMH	Trans Membrane Helices
QSP	Quantitative System Pharmacology
mPTP	Mitochondrial Permeability Transition Pore
CYP3A4	Cytochrome P450 3A4
ATP	Adenosine Triphosphate
TCA	Tricarboxylic Acid
BSG	Basigin
GPF	Grid Parameter File
GSK3β	Glycogen Synthase Kinase 3 Beta
AMPK	AMP-activated Protein Kinase

RXRα	Retinoid X Receptor Alpha
LBP	Lipopolysaccharide-Binding Protein

PENYELIDIKAN TERHADAP INTERAKSI ANTARA STATIN DAN MONOKARBOKSILAT TRANSPORTER 1: SEBUAH PENDEKATAN MOLEKULAR DOKING

ABSTRAK

Statin sering diberikan dalam pengurusan penyakit kardiovaskular; namun, ia boleh menyebabkan gejala otot yang berkaitan dengan statin (SAMS), yang sering dikaitkan dengan disfungsi mitokondria. Transporter monokarbosilat (MCT1) adalah transporter monokarboksilat yang terikat proton yang memudahkan pengambilan statin oleh sel, mempengaruhi farmakokinetiknya dan berpotensi untuk mempengaruhi metabolisme selular dan fungsi mitokondria. Walaupun interaksi langsung antara statin dan MCT1 belum banyak didokumentasikan, bukti yang semakin berkembang menunjukkan bahawa disfungsi mitokondria yang dikaitkan dengan statin mungkin melibatkan mekanisme dimediasi oleh MCT1, berkemungkinan melalui perubahan dalam pengangkutan laktat serta pengawalan metabolik. Kajian ini meneroka interaksi molekul antara statin dan transporter monokarboksilat 1 (MCT1), dengan fokus terhadap kelebihan pengikatannya dan kesan seterusnya terhadap fungsi mitokondria dan pengawalan gen. Struktur 3D MCT1 daripada *Rattus norvegicus* telah dimodelkan menggunakan pangkalan data Swiss-Model, berdasarkan urutan yang serupa daripada *Mus musculus*. Analisis dok molekul, menggunakan kaedah pemautan buta dan khusus, menunjukkan bahawa lakton atorvastatin mempunyai kelebihan keafinitian pengikatan tertinggi pada MCT1 (-8.7 kcal/mol dan -9.2 kcal/mol, masing-masing), diikuti oleh lakton rosuvastatin (-7.5 kcal/mol dan -7.9 kcal/mol), lakton simvastatin (-7.7 kcal/mol untuk kedua-duanya), lakton pravastatin (-7.4 kcal/mol untuk kedua-duanya), dan asid simvastatin (-5.7 kcal/mol dan -6.0 kcal/mol). Daripada semua statin

yang dianalisis, asid simvastatin tidak mempunyai sebarang ikatan hidrogen dengan residu asid amino MCT1, oleh itu boleh menjelaskan kelebihan pengikatannya yang paling rendah. Ini berbeza daripada statin lain. Residue pengikatan penting, termasuk LEU132, TYR70, dan THR388, dikenalpasti sebagai penting untuk interaksi ligan. Dengan mengenal pasti interaksi molekul utama yang menyumbang kepada SAMS, kajian ini mewujudkan rangka kerja yang kukuh untuk ramalan awal penglibatan MCT1 semasa proses patologi.

INSIGHTS INTO THE INTERACTION BETWEEN STATINS AND MONOCARBOXYLATE TRANSPORTER 1: A MOLECULAR DOCKING APPROACH

ABSTRACT

Statins are commonly prescribed in the management of cardiovascular diseases; however, they can lead to statin-associated muscle symptoms (SAMS), which are often related to mitochondrial dysfunction. Monocarboxylate transporter (MCT1) is a proton-linked monocarboxylate transporter that facilitates the cellular uptake of statins, influencing their pharmacokinetics and potential effects on cellular metabolism and mitochondrial function. Although direct interactions between statins and MCT1 are not well-documented, emerging evidence suggests that mitochondrial dysfunction associated with statins may involve MCT1-mediated mechanisms, potentially through alterations in lactate transport and metabolic regulation.. This study explores the molecular interactions between statins and MCT1, focusing on their binding affinities and the subsequent effects on mitochondrial function and gene regulation. The 3D structure of MCT1 from *Rattus norvegicus* was modeled using the Swiss-Model database, based on similar sequences from *Mus musculus*. Molecular docking analyses, employing both blind and specific docking methods, indicated that atorvastatin lactone had the highest binding affinity to MCT1 (-8.7 kcal/mol and -9.2 kcal/mol, respectively), followed by rosuvastatin lactone (-7.5 kcal/mol and -7.9 kcal/mol), simvastatin lactone (-7.7 kcal/mol for both), pravastatin lactone (-7.4 kcal/mol for both), and simvastatin acid (-5.7 kcal/mol and -6.0 kcal/mol). Of all statins analyzed, simvastatin acid does not have any hydrogen bonds with amino acid residues of MCT1 thus could explained its lowest binding affinity. It unlike other

statins. Important binding residues, including LEU132, TYR70, and THR388, were identified as essential for ligand interactions. By identifying the key molecular interactions that contribute to SAMS, this study establishes a solid framework for early prediction of MCT1 involvement during the pathology process.

CHAPTER 1

INTRODUCTION

1.1 Research Background

Statins are among the most prescribed medications worldwide for lowering cholesterol and preventing cardiovascular diseases. They work by inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, an enzyme crucial for cholesterol biosynthesis (Bansal & Cassagnol, 2023). However, despite their benefits, statins can cause side effects, especially in muscles, leading to conditions collectively known as statin-associated muscle symptoms (SAMS) (Wiggins et al., 2022). These side effects, which include muscle pain, weakness, and in severe cases, rhabdomyolysis, are increasingly linked to mitochondrial dysfunction (Schirris et al., 2015). Research indicates that lipophilic statins, such as simvastatin, are more likely to penetrate muscle cells, leading to higher incidences of toxicity compared to hydrophilic statins like pravastatin (Panajatovic, Miljenko, M.P., 2023). Among statins, the lactone forms of simvastatin and pravastatin exhibit distinct pharmacokinetic and pharmacodynamic properties, influencing their cellular uptake and subsequent effects on mitochondrial function.

Monocarboxylate transporters (MCTs), particularly MCT1, are integral to the transport of lactone and acidic drug forms across cellular membranes. *Rattus norvegicus* (rat) MCT1 provides a valuable model for understanding these interactions in skeletal muscle cells, where mitochondrial toxicity is a critical concern. The molecular mechanisms underlying the binding, uptake, and regulation of MCT1 by statins, particularly simvastatin lactone, pravastatin lactone, simvastatin acid, atorvastatin and rosuvastatin remain poorly understood.

The molecular docking approach can be used to mimic the interaction between a small molecule and a protein at the atomic level, allowing us to define small molecule behavior in target protein binding sites while elucidating essential biochemical processes (Meng et al., 2011). Molecular docking is capable of accurately predicting molecules' binding capabilities to a certain target. This coupling can include electrostatic interactions, Van der Waals, Coulombic, and hydrogen bonds. A docking score approximates the sum of the interactions between two structures, indicating their bonding potential. This prediction is made in two steps: first, the algorithm searches the conformational space and presents the probable locations where the molecule could be linked to the target; second, it calculates the energy levels required to achieve the coupling at each of the possible connection points. The lowest energy values are regarded as the most promising for binding (Diego Romário Silva et al., 2019). Knowing the location of the binding site prior to docking operations greatly improves docking efficiency. In many cases, the binding site is already known before docking ligands into it. In addition, information about the sites can be obtained by comparing the target protein to a family of proteins with comparable functions or proteins co-crystallized with other ligands. In the absence of known binding sites, cavity detection tools or online servers such as GRID, POCKET, SurfNet, PASS, and MMC can be used to find potential active sites within proteins. (Meng et al., 2011).

This study aims to investigate the molecular interactions of simvastatin lactone, pravastatin lactone, simvastatin acid, atorvastatin and rosuvastatin with MCT1 in *Rattus norvegicus*, comparing on their differential binding affinities and regulatory effects. By exploring these mechanisms, the research seeks to elucidate the role of

MCT1 in statin-induced mitochondrial toxicity and provide insights into safer therapeutic strategies.

1.2 Research Problem

The prevalence of hypercholesterolemia is on the rise, leading to an increase in the prescription and use of statins as a treatment. While statins are effective in managing cholesterol levels, prolonged use can result in statin-related myopathy (SRM), which can range from mild symptoms like myalgia to severe conditions such as rhabdomyolysis, the most extreme form of muscle disease associated with statins. Notably, different statins exhibit varying levels of toxicity, with lipophilic statins, such as simvastatin, showing a higher incidence of muscle-related side effects compared to hydrophilic statins like pravastatin (Panajatovic, Miljenko, M.P., 2023). The differing pharmacokinetic properties, such as cellular uptake and distribution, are believed to play a crucial role in the severity of these side effects, but the exact molecular mechanisms remain poorly understood (Schirris et al., 2015).

Molecular docking offers a promising approach to investigate the interaction between statins and their target proteins at the atomic level, potentially revealing insights into how structural differences between statins influence their binding affinities and subsequent toxicity. However, there is limited research exploring how the molecular interactions of various statins with proteins involved in drug transport, such as MCT1, contribute to the varying severity of side effects (Meng et al., 2011). Understanding these interactions through molecular docking could provide critical insights into the mechanisms underlying statin-induced toxicity and help identify safer therapeutic strategies.

Therefore, further research utilizing molecular docking techniques is necessary to elucidate the differential binding profiles of various statins, particularly lipophilic and hydrophilic forms, and their implications for the severity of side effects. This research could pave the way for more effective and personalized statin therapies, minimizing adverse effects while maximizing therapeutic benefits.

1.3 Research Questions

1. How do different statin treatments (lipophilic vs hydrophilic statins) affect the binding affinity and interaction patterns with MCT1?
2. Which statin demonstrates the strongest molecular binding affinity to MCT1 thus leading to higher toxicity?
3. What are the key amino acid residues involved in the interaction between MCT1 and statins based on molecular docking analysis?
4. How do structural differences in statins influence their binding modes and stability when interacting with MCT1?

1.4 Research Objectives

General Objectives:

This study aims to investigate the molecular interactions between statins and MCT1 using molecular docking techniques.

Specific Objectives:

1. To compare the molecular binding affinity of simvastatin lactone and pravastatin lactone with MCT1 through molecular docking analysis

2. To compare the molecular binding affinity of simvastatin lactone and simvastatin acid with MCT1 through molecular docking analysis.
3. To compare the molecular binding affinity of rosuvastatin lactone and atorvastatin lactone with MCT1 through molecular docking analysis.

1.5 Research Hypothesis

This study hypothesized that lipophilic statins (simvastatin and atorvastatin) have a higher binding affinity than hydrophilic statins (pravastatin and rosuvastatin). In addition, acid form of simvastatin has a greater binding affinity than its lactone form.

CHAPTER 2

LITERATURE REVIEW

2.1 Statin

Statins are a class of drugs that are frequently recommended to lower cholesterol and lower the risk of cardiovascular disorders such heart attacks and strokes (Almeida & Budoff, 2019). Their mechanism of action involves blocking the activity of the enzyme HMG-CoA reductase, which is essential to the mevalonate pathway, which is the method by which the liver produces vital compounds like cholesterol (Lefer et al., 2001). Statins efficiently lower cholesterol production by inhibiting this enzyme, especially low-density lipoprotein (LDL) cholesterol, which is commonly known as "bad" cholesterol (Reiner, 2013). Reducing LDL cholesterol levels lowers the risk of atherosclerosis and related cardiovascular events by preventing plaque from accumulating in the arteries (Abd & Jacobson, 2011; Massimiliano Ruscica et al., 2022).

There are several different varieties of statins, each with its own potency and properties. Examples of commonly used statins include atorvastatin (Lipitor), simvastatin (Zocor), rosuvastatin (Crestor), pravastatin (Pravachol), and lovastatin (Mevacor). Statins are known for their ability to lower cholesterol and prevent cardiovascular disease. They also serve to stabilize arterial plaque, making it less likely to rupture and lead to heart attacks or strokes (Almeida & Budoff, 2019). Despite their numerous advantages, statins can occasionally induce adverse effects such as muscle soreness and weakness, increased liver enzymes, and digestive problems (Abd & Jacobson, 2011; Massimiliano Ruscica et al., 2022).

One of the most commonly given statins is atorvastatin (Lipitor), which is known for its great potency and efficiency in decreasing LDL cholesterol. Simvastatin (Zocor) is another popular statin known for its moderate potency and cost-effectiveness (Abd & Jacobson, 2011). Rosuvastatin (Crestor) is well-known for its great efficacy in lowering LDL cholesterol and is frequently prescribed to people at increased cardiovascular risk (Almeida & Budoff, 2019). Pravastatin (Pravachol) is a statin with a lower risk of drug interactions due to its unique metabolic pathway, making it ideal for patients on complex prescription regimens (Massimiliano Ruscica et al., 2022). Lovastatin (Mevacor), an early statin, has modest potency and is often prescribed to patients with lower cholesterol reduction demands (Reiner, 2013).

2.1.1 Chemical properties of statins

Statins are classified based on their chemical structures (type 1 and type II), or synthesis; natural, semi-synthetic, and synthetic (Figure 2.1) (Murphy et al, 2020). Lipophilic statins, like simvastatin, atorvastatin, and lovastatin, can easily permeate cell membranes, allowing them to affect both the liver and other tissues in the body. This greater dispersion can improve their effectiveness, but it may also raise the probability of side effects such as muscle soreness and effects on the central nervous system. Hydrophilic statins, such as pravastatin and rosuvastatin, are more focused on the liver and have less adverse effects. While lipophilic statins are commonly chosen for their broad efficacy, hydrophilic statins are typically prescribed for people who are more susceptible to adverse effects.

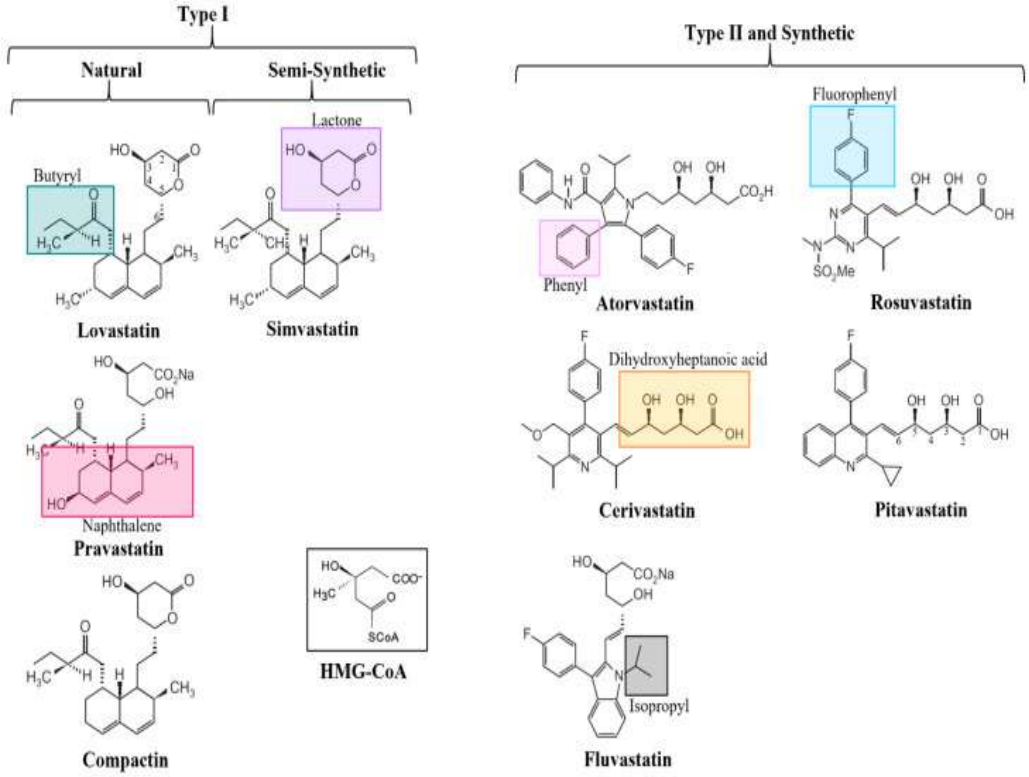

Figure 2.1 Classification of statin drugs (adapted from Murphy et al., 2020)

Simvastatin is one of the commonly used lipid-lowering medications under the statin group and has the molecular formula $C_{25}H_{38}O_5$, its molecular weight being 418.57 g/mol. It is a lactone prodrug hydrolysed *in vivo* to the active β-hydroxyacid form, responsible for its cholesterol-lowering properties. The structure of the compound comprises a lactone ring, a decalin ring system, and a side chain with ester and hydroxyl groups. Such functional groups contribute to its special pharmacokinetic and pharmacodynamic properties. (Istvan & Johann Deisenhofer, 2001). Simvastatin is presented as an active hydroxyacid form with a pKa value of about 4.46, slightly water-soluble –having a water solubility of about 12.7 µg/mL at 25 °C but is freely soluble in organic solvents like ethanol, methanol, and Dimethyl Sulfoxide (DMSO). This poor aqueous solubility requires different formulation strategies aimed at enhancing its

bioavailability. It is stable under acidic conditions, as would be expected from a prodrug, but hydrolyzes to its active form under basic conditions (Corsini et al., 1999).

Pravastatin represents one of the hydrophilic statins and has the molecular formula $C_{23}H_{36}O_7$ and a molecular weight of 424.54 g/mol. Unlike simvastatin, pravastatin is not a prodrug, and it exists in its active β-hydroxyacid form (Corsini et al., 1999). Pravastatin is moderately water-soluble, with a solubility of 1.2 mg/mL at 37 °C, and it demonstrates hydrophilic properties, which differentiate it from more lipophilic statins like simvastatin. This hydrophilicity contributes to its selective hepatic uptake and minimal penetration into extrahepatic tissues, reducing the likelihood of systemic side effects. Pravastatin has a pKa of 4.5, indicating that its carboxylic acid group can ionize in physiological conditions, aiding in its hydrophilic nature (McKenney, 2003). The pharmacokinetic profile of pravastatin is characterized by rapid absorption following oral administration, with a bioavailability of about 17%, due to significant first-pass hepatic extraction. Unlike simvastatin, pravastatin's hydrophilic nature and lower lipophilicity limit its non-hepatic distribution, making it less likely to cross the blood-brain barrier or accumulate in non-hepatic tissues. These properties make pravastatin particularly suitable for patients who may be at higher risk of muscle-related side effects associated with lipophilic statins (Endo, 2010).

Rosuvastatin is a synthetic, hydrophilic and has been regarded as a very effective HMG-CoA reductase (an enzyme that plays a key role in cholesterol biosynthesis) inhibitor based on its strong affinity for the enzyme. Rosuvastatin has the molecular formula $C_{22}H_{28}FN_3O_6S$ with a molecular weight of 481.54 g/mol. It is usually available in the form of rosuvastatin calcium, which is its salt form. The salt improves the stability

and solubility of the compound (Olsson et al., 2002). Rosuvastatin is highly hydrophilic and highly soluble, to 2.1 mg/mL at 25°C. Due to its hydrophilic nature, it undergoes selective hepatic uptake, thus limiting penetration into non-hepatic tissues and hence decreasing systemic side effects. It has a pKa of about 4.6, reflecting its acidic nature, and a partition coefficient Log P of 0.13, showing preference for an aqueous environment. Unlike lipophilic statins such as simvastatin, the hydrophilic nature of rosuvastatin provides a considerable background to its safety and tolerability profile (Corsini et al., 1999). The high potency and hydrophilic nature of rosuvastatin have allowed this agent to be very effective in reducing LDL-C levels while also minimizing the risk of myopathy and other systemic adverse effects. It has a long half-life of almost 19 hours, which allows for once-daily dosing with sustained therapeutic effects. (Martindale: The Complete Drug Reference, 2023).

Lastly, atorvastatin is a synthetic, lipophilic statin and has the molecular formula $C_{33}H_{35}FN_2O_5$ and a molecular weight of 558.64 g/mol. Atorvastatin is generally used as atorvastatin calcium, which improves its solubility and stability (Corsini et al., 1999). Atorvastatin is lipophilic, having a Log P of approximately 5.7, reflecting its strong affinity for the environment of lipids. It is slightly soluble in water, less than 1 mg/mL, and easily solubilizes in organic solvents like ethanol and methanol. Calculated pKa values are 4.46 for the acidic group and 9.37 for the basic group, indicating its amphoteric nature. This would, therefore, suggest that it might have a variable ionization and absorption due to physiological pH conditions. (McKenney, 2003). The lipophilic nature of atorvastatin allows it to permeate hepatocytes efficiently and interact with HMG-CoA reductase, the primary site of action. However, this property also allows it to distribute into extrahepatic tissues, which may contribute to the

potential for systemic side effects, such as myopathy. Atorvastatin undergoes extensive metabolism via the cytochrome P450 enzyme CYP3A4, and its metabolites retain pharmacological activity, further enhancing its lipid-lowering effects (Endo, 2010).

2.1.2 Pharmacokinetics and pharmacodynamics

Statins' active component is a modified 3,5-dihydroxyglutaric acid moiety that is structurally comparable to the natural substrate, HMG-CoA, as well as the mevaldyl CoA transition intermediate. This active site binds to and inhibits HMG-CoA reductase activity in a stereoselective manner that necessitates the statin' s 3R,5R structure. Statins' pharmacokinetic properties vary depending on the form in which they are delivered and their lipophilicity (Ward et al., 2019). Lipophilic statins, such as simvastatin and atorvastatin, are known for their higher prevalence of side effects compared to hydrophilic statins like pravastatin and rosuvastatin. One of the key reasons for this difference lies in the pharmacokinetics of these drugs, specifically their metabolism, elimination, and ability to penetrate cell membranes.

Lipophilic statins are fat-soluble, which allows them to easily diffuse through the lipid bilayers of cell membranes. This capability enables them to penetrate a wide range of tissues beyond the liver, including skeletal muscles, where they are more likely to cause adverse effects. Once inside the muscle cells, lipophilic statins can accumulate, leading to disruptions in cellular processes. For example, they can impair mitochondrial function, resulting in an energy deficit and increased production of reactive oxygen species (ROS). These mitochondrial disruptions are closely associated with muscle-related side effects, such as myopathy, muscle pain, weakness, and in severe cases, rhabdomyolysis (Ahmadi et al., 2018).

In contrast, hydrophilic statins are more water-soluble and exhibit a higher degree of hepatoselectivity. They are primarily taken up by the liver, the main site of their cholesterol-lowering action, via active transport mechanisms involving specific membrane transporters. This selective uptake reduces their distribution to other tissues, including muscle cells, which in turn lowers the risk of side effects. Hydrophilic statins' limited ability to penetrate non-hepatic tissues is a significant factor in their generally safer profile concerning muscle-related adverse effects (Sirvent, Mercier, & Lacampagne, 2008).

The metabolism and elimination of statins further contribute to the differences in their side effect profiles. Lipophilic statins are predominantly metabolized by the cytochrome P450 enzymes in the liver, particularly CYP3A4 for drugs like simvastatin and atorvastatin. This metabolic pathway can be influenced by various factors, including genetic variations, concurrent medications, and dietary components, which may increase the levels of these statins in the blood. Elevated plasma concentrations of lipophilic statins enhance their distribution to non-hepatic tissues, heightening the risk of side effects (Ramachandran & Wierzbicki, 2017).

Moreover, the elimination of lipophilic statins primarily occurs through oxidative biotransformation, followed by biliary excretion. The reliance on metabolic pathways for their clearance means that any factors impairing liver function or interacting with the cytochrome P450 system can lead to an accumulation of the drug in the body, further increasing the potential for adverse effects. On the other hand, hydrophilic statins are less dependent on hepatic metabolism for their elimination. They

are more likely to be excreted unchanged in the urine, which reduces the risk of drug accumulation and associated side effects (Reiner, 2013). This difference in metabolism and elimination further explains why hydrophilic statins tend to have a lower incidence of muscle-related side effects compared to their lipophilic counterparts.

In summary, the higher prevalence of side effects associated with lipophilic statins can be attributed to their ability to penetrate a broader range of tissues, their complex metabolism involving cytochrome P450 enzymes, and their potential for accumulation in the body. Hydrophilic statins, with their more selective hepatic targeting, reduced tissue penetration, and simpler elimination pathways, generally exhibit a safer profile with fewer adverse effects, particularly concerning muscle toxicity.

2.1.3 Mechanism of action of statin

Statins act by competitively inhibiting the active site of the first and most important rate-limiting enzyme in the mevalonate pathway, HMG-CoA reductase as seen in Figure 2.2 (Pulsenotes, 2022). Inhibition of this site restricts substrate access, preventing the conversion of HMG-CoA to mevalonic acid. This lowers hepatic cholesterol synthesis resulting in increased microsomal HMG-CoA reductase production and cell surface LDL receptor expression. This allows for greater clearance of LDL-c from the bloodstream, resulting in a 20% to 55% drop in circulating LDL-C levels.12 Statins may have non-lipid-related pleiotropy effects in addition to lowering LDL-c and cardiovascular morbidity and death. These include enhanced endothelial function, atherosclerotic plaque stabilization, anti-inflammatory, immunomodulatory,

and antithrombotic effects, bone metabolism effects, and a lower risk of dementia. These additional benefits are expected to result mostly from inhibiting the synthesis of isoprenoid intermediates in the mevalonate pathway (Ward et al., 2019).

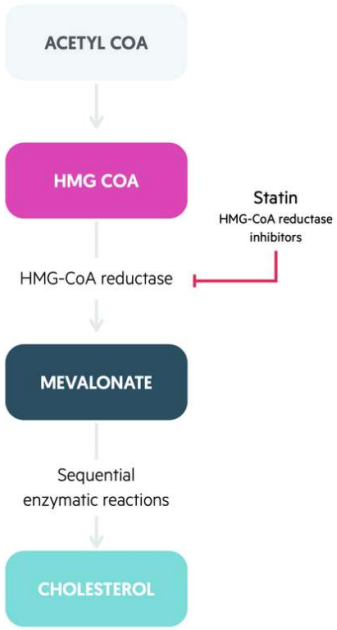

Figure 2.2 Mechanism of action of statin drug in the liver

(adapted from Pulsenotes 2022)

2.1.4 Side effects of statin

Statins, while highly successful in lowering cholesterol and cardiovascular risk, have a number of negative effects. These side effects vary in severity and frequency across patients and can have an impact on their long-term adherence to statin therapy. Muscle-related symptoms are a common and well-documented side effect of statins, including myalgia (muscle pain), myopathy (muscle weakness), and, in rare cases, rhabdomyolysis. Myalgia is characterized by muscle pains or soreness, which can occur

even when creatine kinase (CK) levels are not significantly elevated. Statin-induced myopathy is more severe, with high CK levels causing muscular weakness and pain (Abd & Jacobson, 2011). Rhabdomyolysis, although rare, is a dangerous illness that can cause kidney damage and requires rapid medical intervention (Massimiliano Ruscica et al., 2022).

Another significant side effect of statins is an increase in liver enzymes, including alanine transaminase (ALT) and aspartate transaminase. These enzymes are indicators of liver function, and their elevated levels suggest liver inflammation or injury. While small elevations are normal and generally asymptomatic, large rises may demand statin withdrawal or dosage adjustment (Reiner, 2013). Patients using statins should have their liver function monitored on a regular basis to discover any potential hepatic concerns early on.

Statins have also been linked to an increased risk of developing type 2 diabetes, especially after long-term use. This risk is thought to be related to statins' potential impact on glucose metabolism, as they may modestly raise blood sugar levels (Almeida & Budoff, 2019). The absolute risk is minor, but it becomes more serious in people who already have risk factors for diabetes. Despite this, the cardiovascular advantages of statin medication outweigh the risk of diabetes in the vast majority of patients, particularly those at high risk of cardiovascular events.

There also have been worries regarding statins' potential cognitive adverse effects, such as memory loss and confusion. Although some individuals have experienced similar symptoms, large-scale research have yielded inconclusive findings.

The Food and Drug Administration (FDA) has said that these cognitive side effects are often reversible after stopping the statin, and that the overall risk appears to be minimal (Abd & Jacobson, 2011).

There are also gastrointestinal adverse effects associated with statin therapy such as nausea, constipation, diarrhea, and stomach pain, however they are less prevalent. These symptoms are often mild and temporary, although they might be unpleasant for certain patients, potentially leading to treatment termination (Massimiliano Ruscica et al., 2022).

2.2 Monocarboxylate Transporter

MCTs are known for conveying short chain monocarboxylates such lactate, pyruvate, and butyrate. At present, fourteen members of this transporter family have been identified by sequence homology, with only the first four (MCT1–MCT4) displaying proton-linked transport of monocarboxylates (Halestrap, 2013; Halestrap & Wilson, 2012). Sodium-coupled MCTs (SMCTs) are another transporter family that transports endogenous monocarboxylates. These function as symporters and rely on a sodium gradient for their activity (Felmlee et al., 2020). The isoforms differ in their substrate selectivity and tissue location. Since their expression in the kidney, gut, and brain, these transporters may have a significant impact on drug disposition (Halestrap, 2013; Felmlee et al., 2020). Aside from endogenous short-chain monocarboxylates, they also facilitate the transfer of foreign medicines, including salicylic acid, valproic acid, and simvastatin acid (Halestrap & Wilson, 2012; Felmlee et al., 2020). These transporters serve an important role in cellular metabolism and pH homeostasis,

particularly in tissues that rely largely on glycolysis for energy production, such as muscles and the brain (Felmlee et al., 2020). MCTs contribute to intracellular pH regulation by managing the exchange of lactate and pyruvate, reducing acidosis during periods of high metabolic activity when lactate synthesis rises (Halestrap & Meredith, 2004).

2.2.1 MCT isoforms

MCTs are part of the SLC16 gene family, comprising at least 14 identified isoforms. Each isoform has distinct tissue distribution and substrate specificity, reflecting its physiological role in metabolism and other cellular processes. Firstly, MCT1 (SLC16A1) is ubiquitously expressed, with prominent levels in oxidative tissues such as skeletal and cardiac muscle, as well as red blood cells. It facilitates the transport of lactate, pyruvate, and ketone bodies, crucial for lactate uptake in oxidative tissues and efflux from glycolytic tissues (Halestrap & Wilson, 2012). In cancer cells, MCT1 is overexpressed, promoting lactate uptake to fuel oxidative metabolism, a feature of metabolic reprogramming in tumors (Payen et al., 2020). Next, MCT2 (SLC16A7) primarily found in the brain, liver, and testis, MCT2 exhibits a high affinity for pyruvate and lactate. It is integral to neuronal lactate uptake, supporting brain metabolism and energy homeostasis. Dysregulated MCT2 expression has been linked to neurological disorders, highlighting its importance in maintaining neuronal health (Bergersen, 2007; Simpson et al., 2007).

Not only that, MCT3 (SLC16A8), which predominantly expressed in the retinal pigment epithelium and the choroid plexus. It is essential for transporting lactate and pyruvate in the retina, supporting photoreceptor function and maintaining visual health.

Mutations or dysregulation of MCT3 may result in retinal pathologies (Yoon et al., 1997; Philp et al., 2001). Moreover, MCT4 (SLC16A3) which is expressed in glycolytic tissues such as white skeletal muscle and cancer cells, facilitating the export of lactate to prevent intracellular acidification. Its upregulation in tumours support the Warburg effect, characterized by enhanced glycolysis and lactate production in cancer cells (Ullah et al., 2006; Fisel et al., 2018). This adaptation enables tumour cells to thrive in hypoxic environments.

In addition, MCT8 (SLC16A2). Unlike MCT1–4, MCT8 specializes in the transport of thyroid hormones, such as triiodothyronine (T3) and thyroxine (T4). It is highly expressed in the brain and liver and plays a crucial role in thyroid hormone regulation. Mutations in MCT8 lead to Allan-Herndon-Dudley syndrome, a rare neurological disorder caused by impaired thyroid hormone transport (Friesema et al., 2003). Lastly, MCT10 (SLC16A10) which primarily mediates the transport of aromatic amino acids, including tryptophan and phenylalanine. It is expressed in the intestine, kidney, and liver, influencing amino acid metabolism and associated signalling pathways (Kinne et al., 2010). The proper functioning of MCT isoforms is regulated by accessory proteins, such as basigin (CD147) and embigin, which ensure their appropriate localization and activity (Kirk et al., 2000). Additionally, transcription factors like hypoxia-inducible factor-1α (HIF-1α) upregulate MCT4 expression under low oxygen conditions, highlighting the dynamic regulation of MCT activity in response to metabolic demands (Eales et al., 2016). The diversity in substrate specificity and tissue distribution among MCT isoforms underscores their pivotal roles in cellular metabolism. Understanding their functions provides valuable insights into their

involvement in health and disease, including metabolic disorders, cancer, and neurological conditions.

2.2.2 Chemical structures of MCT

MCT are members of the solute carrier family 16 (SLC16) of proton-coupled transporters, which facilitate the transmembrane movement of monocarboxylates such as lactate, pyruvate, and ketone bodies. They consist structurally of 12 Transmembrane Helices (TMHs), with both the N- and C-termini being intracellular (Figure 2.3). This topology is common to the major facilitator superfamily (MFS) transporters, which utilize electrochemical gradients to drive substrate transport (Halestrap, 2013).

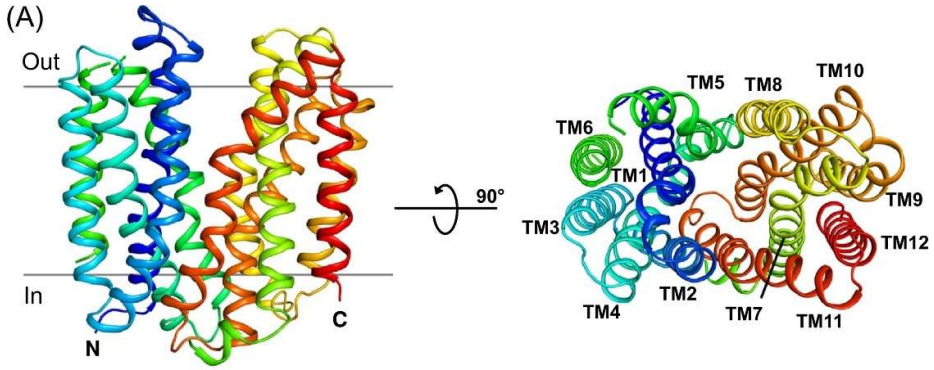

Figure 2.3 Homology model of human MCT1

(adapted from Bosshart et al., 2020)

In MCTs, the TMHs form a central aqueous channel in which substrate binding and translocation take place. The channel allows the transport of polar or charged monocarboxylate molecules across the hydrophobic lipid bilayer. The helices themselves are amphipathic, with polar residues lining the channel to interact with substrates, while hydrophobic residues stabilize the transporter within the lipid bilayer.

The substrate-binding pocket is defined by conserved residues within the transmembrane (TM) domains. For instance, in MCT1, Arg-306 interacts with the carboxyl group of substrates like lactate or pyruvate, while His-385 and Phe-367 contribute to the proton coupling essential for substrate translocation (Wilson et al., 2005). The pocket's hydrophilic nature facilitates monocarboxylate binding, whereas adjacent hydrophobic residues ensure specificity for small monocarboxylic acids (Halestrap, 2013). MCTs operate as proton symporters, coupling substrate transport with proton movement. This is mediated by specific residues, including histidine and aspartate within the TMHs, acting as proton acceptors or donors (Halestrap, 2013).

Peculiar among transporters, MCTs require accessory proteins like CD147/BSG or embigin for stability, trafficking, and localization to the plasma membrane. For instance, CD147 interacts with the extracellular loops of MCT1 and MCT4 to ensure correct membrane insertion. Disruption of these interactions, perhaps due to alterations in the membrane environment induced by statins, could lead to the malfunction of MCTs and further contribute to mitochondrial dysfunction (Kirk et al., 2000).

Subtle differences in the structure of the extracellular and intracellular loops despite the common 12-TMH core in all the MCT isoforms determine substrate specificity and tissue distribution. MCT1 is highly expressed in metabolically active tissues like skeletal muscles and the brain and has a high affinity for lactate and pyruvate (Wilson et al., 2005). MCT2 further increases the affinity for pyruvate and is primarily expressed in neurons; MCT4, adapted for lactate export in glycolytic tissues, exerts low affinity but high transport capacity (Halestrap, 2013).

Because the crystal structures of MCTs are not available at high resolution, homology modelling using related MFS transporters, including the bacterial glycerol-3-phosphate transporter GlpT, has contributed useful information about the structure of MCTs. Computational studies have unravelled some conformational states of MCTs, including inward-open and outward-open conformations during substrate transport (Halestrap, 2013).

2.2.3 Regulation of MCT1 in skeletal muscle and mitochondria

Among these, MCT1 is the most significant isoform, crucial for balancing lactate and pyruvate, two metabolites that are key for cellular energy metabolism. During exercise, anaerobic glycolysis produces lactate, which needs to be efficiently removed from muscle cells to avoid acid buildup. MCT1 aids in this lactate export while also bringing pyruvate into the mitochondria for ATP production. In this way, MCT1 helps maintain cellular pH balance and supports mitochondrial function by ensuring a steady supply of pyruvate, an essential substrate for oxidative metabolism. The expression and activity of MCT1 are affected by various physiological and metabolic factors. Exercise, in particular, has a significant impact on MCT1 expression in skeletal muscle. As muscle activity ramps up, especially during prolonged or intense workouts, the need for lactate transport and pyruvate uptake increases, resulting in an upregulation of MCT1. This adaptive response is crucial for meeting the heightened metabolic demands during physical exertion. Research indicates that chronic endurance training enhances MCT1 expression, particularly in type I muscle fibres, which are more engaged in oxidative metabolism (Juel & Halestrap, 1999; Coles et al., 2004). These adaptations promote more efficient metabolic transitions between anaerobic and aerobic

states, ensuring that lactate is effectively exported and pyruvate is readily available for oxidative phosphorylation.

The regulation of MCT1 expression during exercise involves several signalling pathways. AMP-activated protein kinase (AMPK), a crucial regulator of cellular energy balance, is activated during physical activity and promotes MCT1 expression by stimulating transcription factors like PGC-1α. This factor is essential for driving mitochondrial biogenesis and enhancing the muscle's ability to meet increased energy demands. Additionally, lactate, a byproduct of anaerobic metabolism, contributes to the upregulation of MCT1 expression in response to the stress of exercise (Juel & Halestrap, 1999; Halestrap, 2013). Therefore, exercise not only raises the need for lactate efflux but also prepares the muscle to respond by increasing MCT1 levels. MCT1's role goes beyond just transporting lactate; it also affects mitochondrial function. Pyruvate, once transported into the mitochondria by MCT1, enters the TCA cycle to produce ATP, thereby supporting cellular energy production.

However, if MCT1 function is impaired, this process can be disrupted, leading to a decrease in pyruvate availability for mitochondrial respiration and ultimately affecting ATP production. This dysfunction is particularly concerning in energy-demanding tissues like skeletal muscle, where efficient energy production is vital during exercise. Moreover, reduced MCT1 activity can cause lactate to accumulate in the cytoplasm, resulting in intracellular acidosis, which may impair enzyme activity and further hinder muscle function (Halestrap, 2013; Juel & Halestrap, 1999). The dysregulation of MCT1 has significant metabolic consequences. Insufficient MCT1 function can lead to metabolic issues, such as muscle fatigue and exercise intolerance, especially during high-intensity activities. Impaired lactate export and pyruvate uptake

can obstruct oxidative metabolism and mitochondrial function, contributing to muscle weakness. This is particularly evident in various conditions where MCT1 dysfunction or downregulation is linked to muscle pathologies, including mitochondrial disorders.

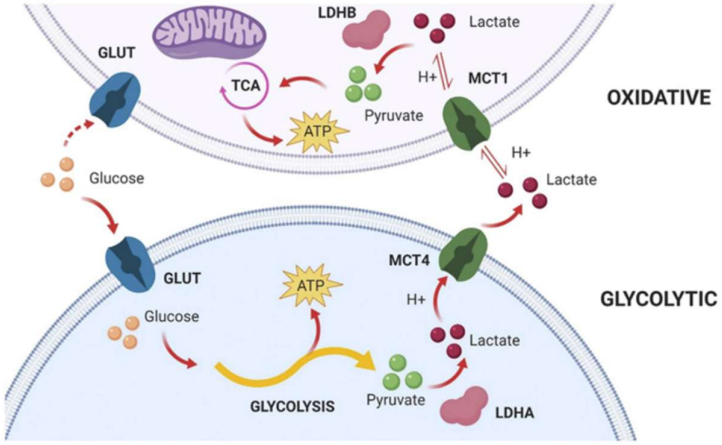

Figure 2.4 Monocarboxylate Transporters (MCTs) and their role in mitochondrial metabolism

(adapted from Felmlee et al., 2020, Fig. 5)

2.2.4 Targeting MCT1 in statin-related mitochondrial dysfunction

Mitochondrial dysfunction is critical in the development of SAMS, as skeletal muscles rely largely on mitochondria for energy production. Statins, which are commonly used to decrease cholesterol levels, can disrupt mitochondrial function via a variety of methods, resulting in muscle discomfort, weakness, and fatigue (Sirvent, Mercier, & Lacampagne, 2008; Ahmadi et al., 2018). Statins primarily influence mitochondria by blocking electron transport chain complexes. Statins can specifically block complexes I, III, and IV, impeding the flow of electrons required for ATP synthesis. Muscle contraction and overall muscle function rely on ATP, therefore any disturbance in its generation can have a substantial influence

on muscle health (Figure 2.4). Furthermore, statins impede the mevalonate pathway, which reduces the synthesis of coenzyme Q10. CoQ10 is an essential component of the ETC, and a lack of it can cause mitochondrial dysfunction and increased oxidative stress, both of which contribute to muscle damage (Marcoff and Thompson, 2007). Another negative side effect of statin treatment is the generation of ROS. Elevated ROS levels produce oxidative stress, which can harm mitochondrial DNA, proteins, and lipids, affecting mitochondrial function (Ahmadi et al., 2018). This oxidative damage worsens muscular complaints and makes muscle cells more prone to injury. Furthermore, statins can affect calcium homeostasis in muscle cells. Statins raise cytosolic calcium levels via altering the mitochondrial permeability transition pore (mPTP) and the Na+/Ca2+ exchanger. This impairs muscular contraction and causes muscle discomfort and weakness (Sirvent, Mercier, & Lacampagne, 2008).

Statins also deplete isoprenoids, such as farnesyl pyrophosphate and geranylgeranyl pyrophosphate, by blocking the mevalonate pathway. Isoprenoids are required for protein prenylation, which is critical for cell structure and function. Their depletion can cause cell death and exacerbate mitochondrial dysfunction (Ramachandran & Wierzbicki, 2017). This multifaceted alteration of mitochondrial functions highlights the intricate link between statin medication and muscle health. Various studies provide evidence that mitochondrial dysfunction occurs in SAMS. Muscle biopsies from SAMS patients frequently show mitochondrial abnormalities, such as lipid buildup and ragged red fibers (Sirvent, Mercier, and Lacampagne, 2008). Biochemical investigations have found aberrant blood lactate/pyruvate ratios and changes in mitochondrial enzyme activity in statin users (Ahmadi et al., 2018).