

Production of Lovastatin and its Lipid-lowering and Anti-Cancer Effects

Yuze Du^{1, †} Yuxiang Liu^{2, *, †} Yunshan Zheng^{3, †}

¹Faculty of Biomedical Science, Monash University, Melbourne, Australia

²Faculty of Agricultural and Environmental Science, McGill University, Montreal, Canada

³Chemistry Department, University of California, Irvine, United States

*Corresponding author. Email: yuxiang.liu@mail.mcgill.ca

†These authors contributed equally.

Abstract. Lovastatin is traditionally used to reduce the amount of cholesterol and lipid levels in many diseases, but its anti-cancer properties are now discovered. By regulating and modulating crucial signaling small G-proteins of cancer cell including Rho, Rac, and Ras, lovastatin can alter cancer cell division, migration, and induce cell death. Lovastatin has a similar structure to HMG-CoA and thus can competitively bind to HMG-CoA reductase (HMGR) and work as a hypolipidemic medicine. The anti-cancer effect of lovastatin had led to extensive research. It had been confirmed based on many in-vitro studies that lovastatin had obvious inhibitory effects on different kinds of cancer. In addition, lovastatin can increase therapeutic effect since it regulates the cell signaling pathway which induces cell cycle arrests. This article covers the application of lovastatin and cancer treatment. Lovastatin has shown promising anti-cancer properties in breast cancers, ovarian cancers and breast cancers, but more evidence is needed to determine its anti-cancer properties in-vivo and in humans.

Keywords: Anti-cancer, Cholesterol, Mechanism, Lovastatin.

1. Introduction

As more and more researchers work on cancer, the understanding of its mechanism and new therapy is growing. Some cancers in their early stages already have robust treatment options with an impressive cure rate, such as mastectomy for breast cancer. However, cancer remains one of the world's top causes of death. Based on the data from the World Health Organization, nearly 20 million new cancers were diagnosed in 2020 and breast cancer accounts for the most. Thus, the search for new anticancer therapies is urgent. At present, relatively mature anti-cancer drug development strategies are mainly based on the discovered targets, that is: through the analysis of the target, develop new drugs or select known drugs for anticancer experiments. Lovastatin is a potential anticancer drug based on the target - small GTPase.

Lovastatin or Monacolin K was first discovered by Alberts, Chen and others in 1978 and it is the first approved hypercholesterolemia medication. The main component of low-density lipoprotein (LDL) cholesterol or LDL-C is cholesterol. LDL-C is related to cholesterol plaque buildup. At high concentrations, LDL-C leads to blood supply problems and a variety of cardiovascular diseases. Lovastatin is known for its effect on cholesterol-lowering and increasing the hepatic uptake of LDL-C by upregulating low-density lipoprotein receptors (LDLR). More and more evidence as well as in vitro and in vivo investigations indicate the anticarcinogenic capacities of lovastatin, such as its novel characteristics in inhibiting cancer cell growth. Today, lovastatin production is highly developed in the industrial world, mainly through fermentation [1].

2. Chemical Structure of lovastatin

Statins class of drug can be classified into three main categories based on the way they are synthesized – natural, semisynthetic, and synthetic. Natural statin drugs are the product of fungal

fermentation. Lovastatin is a natural statin. Semisynthetic statin drug replaces hydrogen on the 2,2-methylbutyryl group of naphthalene ring with the methyl group with the same arrangement of atoms. Both natural and semisynthetic statin drugs share a similar main structure - naphthalene ring - but are different in the substituents. Synthetic statins are produced by chemical synthesis, and the structure is very different from the other two types. The major pharmacophore of statins is dihydroxyheptanoic acid which each type of statin includes – for example, lactone in simvastatin [1].

The chemical structure of lovastatin is composed of a naphthalene ring, hydroxy lactone, a methyl group, and the methylbutyrate group, as shown in Figure 1. The lactone group of lovastatin will open or experience a hydrolysis reaction after intake. The opening lactone group thus has a structure that is comparable to HMG-CoA, which competitively binds and inhibits HMG-CoA reductase (HMGR) to lower cholesterol output [1]. The naphthalene ring is a hydrophobic structure, which is used to mimic the substrate of HMG-CoA. The methylbutyrate group and methyl group can regulate the solubility and hydrophilicity of drugs which is important for pharmacokinetics.

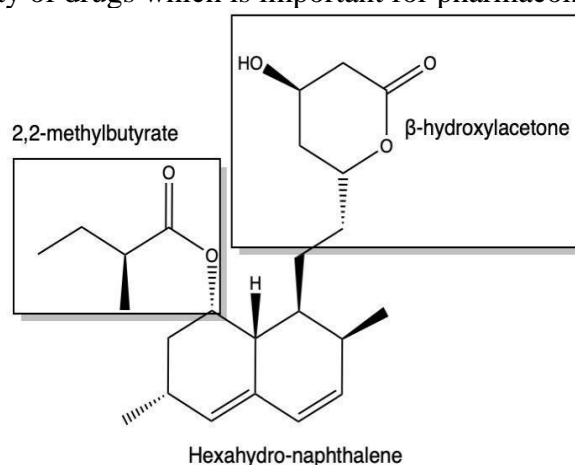


Figure 1 The chemical structure of lovastatin is composed of butyrate moiety, lactone, and naphthalene ring.

3. Production of Lovastatin

Lovastatin can be produced from various filamentous fungal species, but the high-yielding mutants have been derived from *Aspergillus terreus* ATCC 20542, usually through UV light exposure [2].

3.1. Lovastatin Biosynthesis Pathway and Its Biosynthetic Gene Cluster

Figure 2 shows the biosynthetic pathway of lovastatin. There are two branches of lovastatin biosynthesis, and both use malonyl CoA and acetyl CoA as a substrate, which led to the idea of increasing intracellular malonyl CoA through genetic modification of malonate synthetase in ATCC 20542 van den Berg et al. 2009 [3]. Figure 3 shows the 64 kb gene cluster of lovastatin biosynthesis. There are two synthases pertinent to lovastatin biosynthesis: lovastatin nonaketide synthase (LovB) and lovastatin diketide synthase (LovF), and they are encoded by LovB and LovF respectively. The function of these two synthases is to build the carbon skeleton with malonyl CoA and acetyl CoA [2].

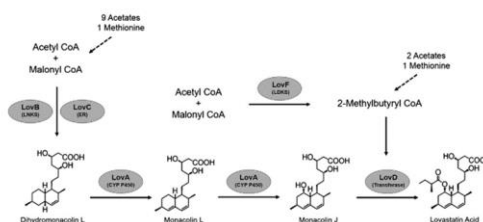


Figure 2 The biosynthetic pathway of lovastatin [2]. Both pathways need malonyl CoA and acetyl CoA as substrates.

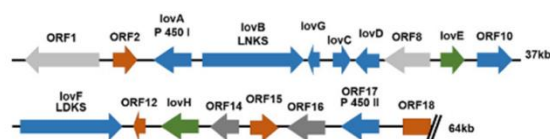


Figure 3 The 64 kb gene cluster of lovastatin biosynthesis [2]. LovB and LovF encode the genes for the two synthases responsible for building lovastatin main skeletons.

3.2. Two Types of Fermentations

The chief production method for lovastatin is fermentation because of its low cost [1]. The two main fermentation processes for lovastatin are submerged fermentation (SmF) and solid-state fermentation (SSF) [2]. For SmF, cultivations of lovastatin-producing microorganisms use a liquid medium with a restricted level of dissolved oxygen, and a slightly acidic pH is required [1, 4]. SmF can be batch fermentation or fed-batch fermentation. Batch fermentation requires the inoculation of microorganisms in a defined volume of culture medium for some time, approximately 10 days in the case of lovastatin production [1]. Fed-batch fermentation gradually “feeds” concentrated nutrients to the medium culture, and fresh medium is continuously added and replaced in a continuous bioreactor [4]. Fed-batch fermentation may be productive, but time-consuming, and contamination-prone, due to the continuous addition and withdrawal of nutrients and broth [4]. In SSF, lovastatin-producing microorganisms are grown on a solid support, usually agricultural waste, such as rice straw, with near-absence of water [2]. SSF mimics the natural environment where filamentous fungi reside [4]. With limited water and non-soluble compounds in the solid culture, fungi activate their genetic machinery to a maximum, producing various enzymes and metabolites for survival [5]. Compared with SmF, the output lovastatin in SSF is more superior, and some secondary metabolites and enzymes are exclusively produced in solid-state fermentation [6].

3.3. Downstream Processing and Extraction

After either submerged fermentation or solid-state fermentation, and their respective downstream processing, lovastatin will be extracted. Submerged fermentation will be followed by acidification, filtration, centrifugation, concentration, and then liquid-liquid extraction, usually using solvent ethyl acetate solvent [2]. The solid-state fermentation process usually uses an organic solvent which varies among protocols [2]. Currently, the identification, quantification, and separation of lovastatin (lactone form and beta-hydroxy-acid form) rely on high-performance liquid chromatography [2].

4. Safety, side effect, and interaction

Lovastatin comes as either an immediate-release tablet or an extended-release tablet. Both tablets are orally taken, and their main difference lies in the release time. Lovastatin is one of the statins, and according to its mechanism of action, it is categorized as HMGCR inhibitor.

Adolescent girls with heterozygous familial hyper-cholesterolemia were studied for the lipid effectiveness of lovastatin medication [7]. In a double-blind, randomized controlled trial, 54 postmenarchal girls were included. After a 4-week diet, patients were randomized into a placebo group or a group treated with lovastatin for 24 weeks. Immediately after 4 weeks of lovastatin treatment, patients received an average reduction of 23% in LDL cholesterol concentration, and a greater reduction at 27% after 24 weeks. At the end of the trial, total cholesterol reduced by 22%, apolipoprotein B reduced by 23%, and triacylglycerol concentration decreased by 23%, with little variation among patients. The study also assessed the safety and tolerability of lovastatin. Overall, it is clear from this study that lovastatin is safe, well-tolerated, and efficacious treatment option for adolescent girls with heterozygous familial hypercholesterolemia. However, more clinical trials should be conducted to assess the safe use of lovastatin in the long run [7].

Lovastatin has side effects. Nausea, heartburn, headache, myopathy, and muscle pain are some of the typical side effects of lovastatin [8]. These side effects are usually mild and tend to disappear in days or a couple of weeks, but serious side effects may occur, such as myopathy, rhabdomyolysis [8].

Lovastatin oral tablets can interact with other medications. As a statin metabolized by CYP3A4, lovastatin should not be taken with CYP3A4 inhibitors, such as Itraconazole, erythromycin and clarithromycin [16]. HIV protease inhibitors such as darunavir and fosamprenavir can induce myopathy while taken with lovastatin [16]. Lovastatin can interact with foods. Grapefruit and grapefruit juice can inhibit CYP3A enzymes and thus interfere with the metabolism of statins [16].

5. Lovastatin and Lipid-lowering

5.1. Lovastatin: Mechanism of Action and Use

Lovastatin is in an inactive form, lactone when orally ingested, and by enzymatic conversion, lovastatin is hydrolyzed to its active form, beta-hydroxyacid in the stomach. The metabolism of lovastatin mainly relies on the CYP3A4 isoenzyme present in the human stomach [9].

Lovastatin is known for its effect on cholesterol-lowering and on increasing the uptake of LDL. Lovastatin can reduce cholesterol synthesis in the liver through competitive inhibition of HMGR. As illustrated in Figure 4, HMGR regulates a key step where mevalonate (MVA) is synthesized in the biosynthetic pathway of cholesterol [10]. This transient inhibition of hepatic cholesterol biosynthesis in turn moves sterol regulatory element binding proteins (SREBPs) to the Golgi [11]. After proteolytic cleavage in the Golgi, the active SREBPs remnant enters the nucleus and switches on genes that regulate cholesterol uptake and biosynthesis, and as a result, upregulates the expressions of HMGR and LDL receptors [11]. Upregulating HMGR would bring cholesterol levels back to normal whereas the presence of more LDL receptors allows hepatocytes to take more LDL and very low-density lipoprotein (VLDL). VLDL, which includes both cholesterols and triglycerides, is converted to LDL-C in the liver, and as VLDL travels through peripheral adipose or muscle tissues and releases triglycerides, VLDL shrinks in size and becomes LDL-C. By increasing the expression of LDL receptors, lovastatin can achieve overall lower levels of LDL cholesterol and triacylglycerol [11]. Because lovastatin act by increasing the expression of LDL receptors, lovastatin is not effective in treating homozygous familial hyper-cholesterolemia where LDL receptors are absent [11].

The reduction of hepatic cholesterol synthesis by lovastatin is beneficial for the prevention of atherosclerosis. Figure 5 shows how LDL cholesterol infiltration and monocyte oxidation in artery walls, the two main causes of atherosclerosis, lead to atherosclerosis [9]. The accumulation of fatty materials like LDL cholesterol in blood vessels can cause atherosclerosis. Chronic fat accumulation in blood vessels can induce LDL cholesterol to oxidize, and this oxidation can result in chronic inflammation. Chronic inflammation in turn leads to the accumulation of cholesterol, as well as plaque formation in blood vessels, and finally, atherosclerosis [12]. Monocytes also participate in the pathophysiology of atherosclerosis. When there is an injury site in the artery wall, monocytes are released in response, and monocytes will infiltrate under the blood vessel cells and trap LDL cholesterols passing by the injury sites, which lead to the formation of foam cells [13]. This thin layer of foam cells in the artery wall is termed fatty streaks. As fatty streaks accumulate and thicken, attracting more muscle cells, lipids, connective tissues and so on, blood vessels and arteries become constricted [13]. Lovastatin can reduce blood LDL-C levels as well as slow progressions of atherosclerosis. Lovastatin also showed its effect in reducing the risks of cardiovascular events [14]. Lovastatin can achieve their anti-atherosclerotic effect through the inhibition of isoprenoids and small GTPase prenylation. The inhibition of isoprenoids leads to many anti-inflammatory effects, including the reduction of monocyte activation [15], a key contributor to atherosclerosis.

According to recent research, lovastatin may be beneficial in combating some types of cancers, such as malignant growths in breast cells and in liver cells [16, 17]. Karampoor et al. reported a putative function for lovastatin in attenuating COVID-19 in a clinical trial published in 2021, such as the reduction of inflammatory markers, overall shorter hospitalization, and lower mortality rate, but

more clinical trials should be conducted before establishing the protective function of lovastatin against COVID-19 [18].

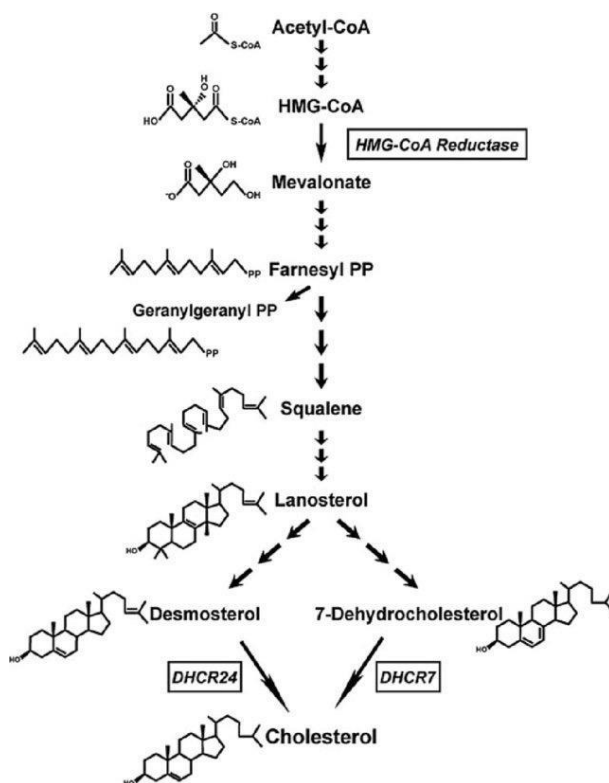


Figure 4 Cholesterol biosynthetic pathway [19]. HMGR regulates an irreversible step in biosynthetic pathway of cholesterol. Lovastatin can inhibit HMGR and thus achieve cholesterol-lowering effects.

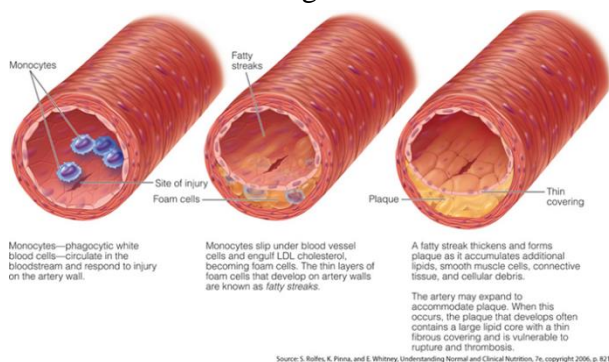


Figure 5 Pathophysiology of atherosclerosis [13]. Monocyte infiltration and LDL cholesterol accumulation in the injury site of artery walls lead to the formation of foam cells and plaques, inflammations, and eventually, atherosclerosis.

5.2. Hypercholesterolemia Treatment

Familial hypercholesterolemia is a genetic metabolic disorder, while primary hypercholesterolemia can be induced by various environmental factors and poor dietary habits. Increased levels of cholesterol products, particularly LDL-C and Apolipoprotein B (ApoB), characterize hypercholesterolemia [11]. Higher LDL cholesterol concentration can cause the formation of foam cells, which further leads to plaque accumulation and atherosclerosis. Atherosclerosis is considered the most important contributing factor of cardiovascular diseases [20].

Lovastatin is the first FDA approved HMGR inhibitor applied in therapies of primary and familial hypercholesterolemia. The function of lovastatin in lowering levels of cholesterol had been evaluated by clinical trials. In a controlled and randomized trial, Evan et al collected 132 patients with age 10-

17 and provided a placebo and lovastatin. The results show the efficacy and safety of lovastatin for lowering cholesterol level for male patients around 10-17 years old [21].

6. Lovastatin and Cancer Therapy

Many forms of malignancies have abnormalities of lipid metabolism and the MAV pathway. Statins drug can change and inhibit the mevalonate pathway by interacting with HMGR. Inhibitions of mevalonate system not only reduce cholesterol, but also inhibit isoprenoid production. Isoprenoids are indispensable for prenylations and activations of Ras and Rho proteins, which are essential players in cancer progression [22]. Also, Ras and Rho proteins belongs to the GTPases superfamily, which is participated in the proliferation and migration of tumor cells [23]. This chapter discusses the potential of lovastatin as an anti-cancer drug in different types of cancer.

6.1. Ovarian Carcinoma

Ovarian cancer is not common, but it is usually diagnosed at advanced stages due to imperceptible symptoms. As a result, ovarian cancer patients have an overall low survival rate. New therapeutics for ovarian cancers are desperately needed [24]. There is a small range of pharmacological alternatives to use, but statins have demonstrated promising features in treating or/and assist in suppressing ovarian cancer cells, even though such anti-cancer effect remains controversial. Lovastatin, like other statin medicines, has been demonstrated to increase apoptotic activities of cancer cells in a tumor-specific way by inhibiting HMGR. According to findings, lovastatin induces apoptotic activities in a p53-independent way. Lovastatin can work together with doxorubicin. Lovastatin kills tumor cells by inhibiting HMGR activity and sensitizes multidrug-resistant cells to doxorubicin in an MVA-independent manner. This clinical trial supports the complementary use of lovastatin with chemotherapeutics to facilitate ovarian tumor cell death [25].

In summary, statins can induce cancer cell deaths through multiple mechanisms, and lovastatin may be useful in the combat against ovarian tumor. Lovastatin is capable of inhibiting inflammation, angiogenesis, and the proliferation of ovarian cancer cells to induce apoptosis.

6.2. Breast Cancer

Breast cancer is characterized by uncontrolled breast cell growth. While breast cancer can be found in both sexes, breast carcinoma is more frequently diagnosed among females, and it ranks second in cause of cancer death in females worldwide [26]. The common treatment of breast cancer in the early stage is surgery. However, for advanced breast cancer, there is a lack of effective and complete treatment, and the existing therapy mainly focuses on relieving symptoms and cancer prevention. Also, although surgical treatment is now well developed, its effect on the psyche of most patients is indelible. Thus, finding more effective medication is still essential.

Statin drug had been proved to inhibit the reproduction of cancer cells and induce their apoptosis and necrosis activities. Paola Cafforio's study in 2005 showed that statins trigger through their internal pathways involving mitochondria [27]. The performance of statins in various experimental models is sufficient to demonstrate their great potential as an anticancer drug. A proteo-metabonomic study evaluated the impact of concentrations of lovastatin on breast cancer cells, it used two common cell lines of breast cancer cells – MDAMB468 and MDAMB231 – to test the relationship between lovastatin concentration and cancer cell proliferation. The results showed that lovastatin successfully inhibited the proliferation of breast carcinoma. Also, A series of subsequent experiments revealed that lovastatin act by affecting Rho and other small GTPase, E2F, and AKT signaling pathways in the cancer cells [16].

Another study investigated the correlation between lovastatin and breast cancer prevalence, it collected the information on health conditions from 25992 aged-matched females including 4332 breast cancer patients using Charlson Comorbidity Index to analyze [28]. The results suggested that

lovastatin was related to lower risk of breast cancer [28]. While this correlation needs careful interpretations, the study provides a study direction of preventing breast cancer.

6.3. Liver Cancer

There is no therapeutic treatment available for liver cancer. Recently, uses of statins to prevent or combat liver cancer became a popular field of study. In-vitro Study by Wang et al. demonstrated that lovastatin may suppress tumors by acting on HepG-2 cells and inducing cell death. Lovastatin utilizes two pathways to induce HepG-2 cells apoptosis. Through activating the mitochondrial apoptotic pathway, lovastatin activates Caspase-3 and increases Bax protein gene transcriptions, and lovastatin reduces the transcription of Bcl-2 protein that prevent cancer cell death. ATF-4 and CHOP are the two markers of endoplasmic reticulum stress-related apoptosis [17]. Lovastatin can upregulate ATF-4 and CHOP through this endoplasmic reticulum stress pathway [17]. In a network meta-analysis, different statins were given to patients with hepatocellular carcinoma, and results showed that statins including lovastatin effectively helped with the prevention of liver cancer [29]. A meta-analysis in 2014 also showed a positive risk reduction of liver cancer by using statins, but it only recommended statins as an “adjuvant” in treating liver cancers due to possible statin-induced liver injury [30]. In conclusion, correlation study shows that lovastatin may reduce risks of liver cancer. More studies are needed before using lovastatin as a treatment for liver cancer.

7. Conclusion

Lovastatin is a hypolipidemic medicine drug that acts by inhibiting HMGR in the cholesterol biosynthetic pathway and upregulating LDL receptors. Primary uses of lovastatin are to treat hypercholesterolemia and heart disease patients, but recent research has found lovastatin helpful in treating various cancers and even against COVID-19. Different filamentous fungal species can produce lovastatin. *Aspergillus terreus* is the main fungal species in the industrial production of lovastatin, with ATCC 20542 being the model strain. Biosynthetically modified yeast and bacteria can also synthesize lovastatin. The industrial production of lovastatin favors fermentation due to its low cost. After either solid-state fermentation or submerged fermentation, cultures would undergo downstream processing and extraction. Lovastatin would then be quantified and separated through HPLC. In conclusion, the industrial production process of lovastatin is highly developed, but there is more to be explored in its application. The role of lovastatin in fungi remains unclear, but recent studies found the antimicrobial property of lovastatin, which could be its very fungal function.

There is a significant endeavor by researchers to investigate the potential anti-cancer properties of lovastatin, especially on ovarian cancers, breast cancers, and liver cancers. As evidenced in various experimental models, statins are promising anti-cancer drugs. Lovastatin induces the apoptosis of ovarian cancer cells by inhibiting cancer cell reproductions, inflammation, and angiogenesis. Statins are now integrated into the chemotherapy to better target ovarian tumor cells and induce their apoptosis. Lovastatin inhibits malignant growths in breast cells either through internal mitochondrial pathways or through AKT signaling pathways, and studies have revealed the preventative effect of lovastatin against breast cancers. Lovastatin can suppress liver cancer cells by inducing the apoptosis of liver cancer cells, either through a mitochondrial apoptotic pathway or endoplasmic reticulum stress-related apoptosis. The preventative effect of lovastatin is supported by two meta-analyses. However, for all types of cancers, the anti-cancer effect of lovastatin on human bodies remained unclear, and more clinical trials are needed before using lovastatin as an anti-cancer drug. The future looks bright for lovastatin, and I expect to see more clinical applications of lovastatin.

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