Berberine: A Multi-Target Natural PCSK9 Inhibitor with the Potential to Treat Diabetes, Alzheimer’s, Cancer and Cardiovascular Disease

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Introduction
Cardiovascular disease (CVD) is the leading cause of death worldwide. The total number of cardiovascular disease cases nearly doubled from 1990 to 2019 from 271 million to 523 million.¹ One of the main treatments for hyperlipidemia and CVD is a reduction in low density lipoprotein cholesterol (LDL-C). Multiple drugs are available on the market that reduce LDL-C including statins, proprotein convertase kexin/subtilisin type 9 (PCSK9) inhibitors, and ezetimibe. Statins are the most widely prescribed drug in this class. Moderate statin therapy has been shown to reduce LDL-C levels by 30-45%, while high-intensity statin therapy can provide reductions of over 50%.² However, some patients, such as those with familial hypercholesterolemia (FH), cannot reach target LDL-C goals with statin therapy alone. Both the American Heart Association and the European Atherosclerosis Society recommend ezetimibe and PCSK9 inhibitors as secondary treatments for at-risk patients with insufficient LDL-C reductions.³

An alternate approach to cholesterol reduction involves using natural products, which are often less costly than their synthetic counterparts and tend to have fewer side effects. The natural compound berberine (Figure 1) is of particular interest for several reasons. First, it lowers cholesterol via multiple pathways.⁴⁻⁹ Furthermore, it attenuates inflammation associated with CVD,¹⁰,¹¹ Alzheimer’s disease (AD),¹²⁻¹⁴ and diabetes¹⁵,¹⁶ by reducing pro-inflammatory cytokines. It is also effective in treating cancer cells¹⁷,¹⁸. This suggests that it could improve health outcomes for multiple diseases simultaneously.
The genus *Berberis* represents the leading and most widely distributed natural source of berberine. Berberine is present in traditional Chinese medicinal plants including *Coptis chinensis* and *Rhizoma copidis* and is believed to have been isolated for the first time from the North American herb turmeric (*Hydrastis canadensis*). Plants in the genus *Berberis* have been used in traditional Chinese and Ayurvedic medicine for over 3000 years to treat inflammation, infections, diarrhea, constipation and to help wound healing. Berberis *vulgaris*, the most widely known species, is native to Europe, Northern Africa, and Asia. Berberine can be found in its stems, roots, bark, and rhizomes. *B. vulgaris* was used as a traditional medicine in Iran, France, Turkey, Bulgaria, and Argentina; people used it to treat many ailments including rheumatoid arthritis, hepatitis, intestinal ulcers, malaria, and as an antihypertensive. Furthermore, berberine has been studied as a treatment for diabetes, cancer, hyperlipidemia, congestive heart failure, and HIV because of its antibiotic, anti-inflammatory, and antineoplastic properties.

**Pharmacokinetics, Bioavailability, and Metabolites**

Pharmacokinetics involves the absorption, distribution, metabolism, and excretion of drugs by the body. Berberine’s main drawback is its reduced bioavailability after oral administration. Its rigid planar structure and quaternary ammonium unit make it mostly insoluble in water. This causes poor absorption in the gastrointestinal (GI) tract. In rats, berberine undergoes extensive first-pass elimination. With intestinal first-pass elimination in the GI tract quantified at 99.5%, it was estimated that only 0.2% of the oral dose enters the portal vein. Using intraportal dosing, Liu et al. determined the hepatic bioavailability of berberine to be 71.8%. Furthermore, intragastric dosing revealed only 44% of berberine was absorbed within 36 hours of administration. This data allowed them to calculate a total bioavailability of 0.37% and a gastrointestinal bioavailability of 1.16%.

Tissue distribution of berberine in rats indicates it is widely distributed throughout the liver, kidneys, muscle, lungs, brain, heart, and pancreas. Berberine levels were higher in tissue samples than plasma four hours after administration for the majority of tissues mentioned. Excretion was measured in rats administration 200 mg/kg. After 48 hours, 23% of the administered dose was found in feces, with trace amounts (0.1%) found in
bile and urine. Of the 23 percent, 3.7% were berberine metabolites. Human recombinant cytochrome P450 (CYP) enzymes were used to determine the CYPs involved in berberine phase I metabolism. The study found CYP2D6 to be the main CYP, followed by CYP1A2 and CYP3A4. CYP2D6 and CYP1A2 convert berberine into demethylated and demethylenated compounds, although CYP1A2 metabolizes berberine at a slower rate than CYP2D6. CYP3A4 is only present in demethylenation.

Figure 2: The phase I metabolites of berberine

Berberine is metabolized in the liver via oxidative metabolism, yielding six major phase I metabolites: demethyleneberberine, berberrubine, jatrorrhizine, thalifendine, columbamine, and dihydroberberine (Figure 2). These metabolites act on the same targets as berberine but with reduced potency. Berberine is not completely transformed into its metabolites during hepatic phase I metabolism. In rats, measuring metabolite concentration three hours after oral administration of 200mg/kg of berberine resulted in a majority of berberine being conserved (~25ng/g liver) and varying concentrations of four metabolites, with thalifendine having the highest prevalence (~4ng/g liver). Berberrubine was present in the smallest quantity at less than 1ng/g liver. The other two metabolites detected were jatrorrhizine and demethyleneberberine. Although this study was conducted with rats, all four metabolites are also found in humans following berberine consumption and the pathways are thought to be similar.

Each metabolite has different properties, which may contribute to berberine’s ability to ameliorate different diseases. Berberrubine is a potent antioxidant and radical OH scavenger. Demethyleneberberine has hepatoprotective effects; it can attenuate cellular damage caused by alcohol consumption and reduce nonalcoholic fatty liver disease. Jatrorrhizine has neuroprotective properties in rats due to the molecule’s inhibition of apoptosis and antioxidant properties. Columbamine suppresses the migration, proliferation, and invasion of colon cancer cells and promotes their apoptosis. Of the four phase I metabolites found in rats, only berberrubine and thalifendine were found to increase low density lipoprotein receptor (LDLR) messenger RNA (mRNA). Berberrubine was shown to have 35% of berberine’s activity while thalifendine had 26%. AMPK plays a key role in lipid, glucose, and protein metabolism. The regulation of AMPK phosphorylation is believed to contribute to berberine’s triglyceride-lowering properties. Berberine, berberrubine, and thalifendine enhanced AMPK alpha phosphorylation in HepG2 cells treated with 20 μM for 24 hours, albeit berberine did so to a higher degree. Demethyleneberberine and jatrorrhizine had no effect. A similar experiment by Cao et al. showed that berberine and columbamine decreased triglyceride (TG) levels by 65%.
Berberine as a Multi-Target Drug Against Diabetes, Atherosclerosis, Cancer, and Alzheimer’s

Berberine (5,6-dihydro-9,10-dimethoxybenzo[g]-1,3-benzodioxolo[5,6-a] quinolinium) is an isoquinoline plant alkaloid with potent pharmacological effects. There is an inverse relationship between berberine consumption and cancer. It acts as an anti-cancer agent against multiple types of cancer by scavenging free radicals, inhibiting angiogenesis, generating reactive oxygen species (ROS), and inducing apoptosis in various cell lines (Figure 3). Berberine downregulates the JNK pathway in pancreatic cancer cells, which results in reduced mRNA levels of TNF-α, IL-1β, and IL-6. Berberine was found to suppress tumor growth in gastric cancer cells via downregulation of the mammalian target of rapamycin (mTOR), phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT), and mitogen-activated protein kinase (MAPK) pathways, which induced autophagy. It modulates several other cell signaling pathways involved in cancer, including the adenosine monophosphate-activated protein kinase (AMPK), and signaling pathways. Berberine also downregulates nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) expression, resulting in reduced inflammation. It inhibits the sterol regulatory element-binding protein (SREBP) cleavage-activating protein (SCAP), which inactivates SREBP1 and cholesterol synthesis. Berberine prevents tumor metastasis and growth by upregulating microRNA 145 (miR-145), which targets and silences numerous oncogenes. It also binds to and downregulates the expression of the hairy/enhancer-of-split transcription factor, which is implicated in metastasis. During epithelial-mesenchymal transition, berberine reduces the prevalence of proteins involved in metastasis including E-cadherin, β-catenin, and cyclin D1. In addition, berberine has cancer-preventative properties and enhances the efficacy of cancer drugs.

Figure 3. Berberine induces apoptosis and prevents the metastasis and tumor growth of cancer cells in vitro via multiple mechanisms. It regulates several signaling pathways including the AMPK, JNK, JAK2/STAT3, COX-2, MAPK, SCAP/SREBP1, and EGFR pathways. It reduces inflammation by downregulating NF-κB and the production of inflammatory cytokines. It also increases the efficacy of cancer drugs via ROS generation and free radical scavenging.
Diabetes is characterized by chronic low-grade inflammation, hyperglycemia, and insulin resistance. Berberine improves Type 2 Diabetes Mellitus (T2DM) in multiple models. A human trial with 36 adults found 500 mg of berberine administered thrice daily for three months to be as effective as metformin. However, 34% of subjects experienced transient gastrointestinal adverse events. In HepG2 cells, berberine increases insulin sensitivity by increasing glycogen synthesis and reducing proinflammatory cytokine levels. It induces glycosylation by activating the AMP-activated protein kinase (AMPK) pathway and suppresses gluconeogenesis. Berberine reduces inflammation by decreasing pro-inflammatory cytokines such as interleukin-6 (IL-6), interleukin-1β (IL-1β), toll-like receptor 4 (TLR4) and tumor necrosis factor-α (TNF-α). Increased levels of these cytokines are linked to Type 2 Diabetes and Alzheimer’s disease. IL-1β and TLR4 contribute to insulin resistance. TLR4 expression is significantly elevated in insulin-resistant mice and humans with impaired glucose tolerance or T2DM. Similarly, high IL-6 levels can predict T2DM. Antagonists of IL-6 tend to have unwanted side effects, such as increased infections, due to the central role of IL-6 in innate immunity. As an antimicrobial, berberine might be able to reduce inflammation without incurring some of these unwanted effects.

Alzheimer’s is a neurodegenerative disease with no current cure. In addition to amyloid beta (Aβ) plaque deposits, AD is characterized by neurofibrillary tangles (NFTs) of hyperphosphorylated tau proteins and inflammation in the brain, which eventually lead to cognitive decline and dementia. One’s chance of developing AD depends upon a set of factors including age, lifestyle, genetics, and comorbidity. Hypertension, obesity, and T2DM are all risk factors for developing AD. IL-1β and TLR4 are also implicated in CVD. Increased TLR4 levels are linked to obesity. Interleukin-6, TNF-α, IL-1β and TLR4 are also implicated in CVD. Increased TLR4 levels are linked to obesity. Interleukin-1 promotes atherosclerotic plaque formation; blocking it improves acute myocardial infarction and ischemic stroke outcomes. Increased TNF levels are associated with higher cardiovascular disease risk and coronary artery disease. IL-1β induces systemic inflammation at sub-nanomolar concentrations. Its genetic expression is low in healthy individuals but increases when disease is present. It is part of a positive feedback loop that can induce its own gene expression. This can lead to a cascade of pro-inflammatory responses. In fact, IL-1 can induce the expression and synthesis of several hundred secondary inflammatory compounds.

Hypercholesterolemia is a major risk factor for cardiovascular diseases such as coronary artery disease, peripheral artery disease, and atherosclerotic cardiovascular disease. Metabolic syndrome and diabetes are also major risk factors. Serum cholesterol reduction is correlated with less cardiovascular complications and is the main therapeutic strategy for reducing CVD risk. Berberine has been found to reduce LDL-C, triglycerides, and total cholesterol in both human and animal studies. In a clinical trial, 500 mg of berberine given with food, daily for 4 weeks reduced LDL-C, total cholesterol, triglycerides, and apolipoprotein B (apoB) by 20%, 16%, 22%, and 15%, respectively.

**Lipid-Lowering Effects**

Berberine reduces lipids through multiple, distinct mechanisms. Berberine stabilizes and upregulates LDLR mRNA, inhibits PCSK9 expression, reduces intestinal cholesterol absorption, and promotes its excretion into the bile, where it is degraded. Berberine has been shown to affect acyl-coenzyme A (acyl-CoA) cholesterol acyltransferase (ACAT). There are two types of acyl-CoA cholesterol acyltransferases: ACAT1...
and ACAT2. ACAT1 is ubiquitous, while ACAT2 can only be found in hepatocytes and intestinal cells. ACAT is essential to cholesterol transport and absorption; it converts cholesterol into a cholesteryl ester. This process contributes to maintaining cholesterol homeostasis and is regulated mainly by ACAT2. Berberine downregulates ACAT2 mRNA and protein levels without affecting ACAT1 levels in Caco-2 cells and the small intestines of rats. Berberine also reduced cholesterol permeability in Caco-2 cells. Reduced intestinal absorption of cholesterol in turn lowers plasma cholesterol levels. Berberine has also been shown to reduce blood cholesterol levels by promoting cholesterol excretion from the liver into the bile in hyperlipidemic hamsters. Hamsters in the control group which were fed a normal diet and given berberine showed no significant differences in cholesterol levels in the liver and bile.

In addition, berberine inhibits PCSK9. PCSK9 plays a vital role in LDLR recycling, and its inhibition reduces serum low density lipoprotein cholesterol levels. When PCSK9 binds to LDLR, the ligand-receptor complex is internalized via endosomes and placed in an acidic lysosome (Figure 4). Acidic conditions strengthen the PCSK9-LDLR interaction, preventing the LDLR from being recycled to the cell surface. Both PCSK9 and LDLR are then degraded. After internalization there is little to no LDLR expression, resulting in a prolonged inability to uptake LDL particles. Consequentially, PCSK9 inhibition promotes LDL-C clearance.

Figure 4: Hepatic PCSK9-LDLR interactions prevent LDLR from being recycled to the cell surface. Instead, both are internalized and degraded in the lysosome.

In tests with human hepatoma cells, berberine not only upregulates LDLR expression but also inhibits the expression of PCSK9 through the accelerated degradation of hepatocyte nuclear factor 1 (HNF1α) proteins.
without affecting \( \text{HNF1}\alpha \) expression.\(^7\) HNF1\(\alpha\) plays a key role in lipid homeostasis and \( \text{PCSK9} \) expression. HNF1\(\alpha\) binds to the \( \text{PCSK9} \) promoter, activating \( \text{PCSK9} \) transcription. In contrast, \( \text{HNF1}\alpha \) knockout mice displayed a 50% reduction in liver \( \text{PCSK9} \) mRNA and serum \( \text{PCSK9} \) levels.\(^94\)

Berberine also increases LDLR expression and mRNA stabilization.\(^92,95\) Berberine increases LDLR promoter activity via activation of the c-Jun NH(2)-terminal kinase (JNK) pathway\(^95\) and increases LDLR mRNA by 300% in HepG2 cells via activation of the extracellular signal-regulated kinase (ERK) signaling pathway. Berberine also prevents LDLR mRNA decay via ERK activation.\(^4,9\) In addition, the increased LDLR mRNA stability produced by berberine treatment was found to be transcriptionally independent and caused by the binding of the proteins hnRNP I and KSRP to three AU-rich elements in the untranslated region (UTR) of LDLR mRNA.\(^96\)

**PCSK9 and EGF-A Interactions**

The LDLR’s epidermal growth factor (EGF) domain plays a vital role in PCSK9-LDLR binding. As such, it has become an important potential target site for blocking PCSK9-LDLR interactions.\(^97\) The EGF precursor domain contains two cysteine-rich EGF-like domains, EGF-A and EGF-B. They are separated from a third EGF-like domain (EGF-C) by a beta-propeller domain. The EGF domain allows LDLR to dissociate from lipoproteins in the endosome after they are internalized.\(^98\)

PCSK9 binds to the first epidermal growth factor-like repeat (EFG-A) of the LDL receptor. The EGF-A domain contains a coordinated \( \text{Ca}^{2+} \) ion which has been shown to play a role in PCSK9-LDLR binding. Several amino acid interactions between PCSK9 and LDLR have been documented, resulting in a 20 Å protein-protein interaction interface. The D310 side chain of LDLR chelates the \( \text{Ca}^{2+} \) ion and forms a salt bridge with R194 of PCSK9, thus mediating electron sharing between LDLR’s \( \text{Ca}^{2+} \) ion and R194. The backbone of D310 interpenetrates the T377 backbone of PCSK9. At the same time, the N295 of LDLR chelates the \( \text{Ca}^{2+} \) ion and also forms a hydrogen bond with D238 of PCSK9. Interestingly, a LDLR mutant known to cause familial hypercholesterolemia, H306Y, shares a phenolic proton with D374 of PCSK9, lending credibility to the idea that there is a strengthened PCSK9-LDLR interaction in those with FH.\(^93\)

The binding strength of PCSK9 to EGF-A increases with lower pH. This ensures that PCSK9 does not become dislodged from the LDLR after internalization in the endosome. PCSK9/EGF-A binding also interferes with the acid-dependent conformational change in LDLR required for receptor recycling. Studies using monkey kidney cells have shown that PCSK9 cannot bind to LDLR in the absence of EGF-A. They concluded that the coordinate calcium binding sites of EGF-A include the amino acids Asn\(^{295}\), Glu\(^{296}\), Asp\(^{310}\), and Tyr\(^{315}\) (Figure 5). Amino acid substitutions were made to determine which ones interfere with PCSK9-LDLR binding. An E296D substitution did not interfere with binding, which demonstrates that Glu\(^{296}\) does not directly influence EGF-A and PCSK9 interactions. When glutamate replaced aspartate at position 310, PCSK9-receptor binding was inhibited.\(^98\) This indicates that PCSK9/EGF-A binding disruption is an effective form of PCSK9 inhibition. Several research groups are already exploring this mechanism in attempts to discover small molecule PCSK9-LDLR disruptors.\(^99–101\) One group has identified a peptide that disrupts the interaction \( \text{in vivo} \),\(^102\) while another has developed an orally bioavailable small molecule which reduced total plasma cholesterol by up to 57% in APOE*3-Leiden.CETP mice.\(^103\)
Mechanism of Action of PCSK9 Inhibition

Berberine is a quaternary protoberberine alkaloid that contains carbon, hydrogen, oxygen, and a positively charged nitrogen.\textsuperscript{20} Its mechanism of action for PCSK9 inhibition is a decrease in PCSK9 transcription caused by a reduced quantity of the HNF1\textsubscript{α} protein, which is necessary for PCSK9 mRNA transcription. HNF1\textsubscript{α} and SREBP control the amount of PCSK9 synthesized in liver tissue. SREBP upregulates PCSK9 gene transcription when intracellular sterol levels are low. An HNF1\textsubscript{α} binding site is located inside the PCSK9 promoter, between the serum response element (SRE) and Sp1 site. The HNF1\textsubscript{α} binding site operates as a tissue-specific cis-regulatory sequence of the PCSK9 promoter through HNF1\textsubscript{α} transcription factor binding.\textsuperscript{18}

The theory that berberine inhibits PCSK9 by accelerating HNF1\textsubscript{α} degradation is corroborated by data showing that mRNA expression of HNF1\textsubscript{α} and SREBP-2 remained unchanged, leading to the conclusion that berberine affects HNF1\textsubscript{α} at the translational level.\textsuperscript{7,104} An \textit{in vivo} study with dyslipidemic mice demonstrated that a 200mg/kg daily berberine treatment reduced PCSK9 serum levels by 50\% and HNF1\textsubscript{α} protein levels by 42\% compared to control mice. HNF1\textsubscript{α} mRNA were not reduced, but LDLR protein levels increased by 67\%. There was enough data to conclude that the reduction of HNF1\textsubscript{α} caused an attenuation in PCSK9 gene expression. The same study elucidated the underlying mechanism involving HNF1\textsubscript{α}. Berberine accelerates the degradation of HNF1\textsubscript{α} proteins via an ubiquitin proteasome system (UPS). In this system, proteins are targeted for degradation by ubiquitin labeling. The proteasome then enzymatically breaks down the labeled proteins. As expected, blocking UPS resulted in the accumulation of HNF1\textsubscript{α}.\textsuperscript{7}

FDA-Approved PCSK9 Inhibitors

There are currently three Food and Drug Administration-approved PCSK9 inhibitors on the market:
evolocumab, alirocumab, and inclisiran. Evolocumab and alirocumab are monoclonal antibodies (mAbs) that require biweekly or monthly injections. They are both well tolerated with minimal side effects and have been shown in a myriad of trials to reduce LDL-C by about 60% when used in conjunction with statin therapy. However, monoclonal antibody treatment is quite costly for patients. In 2015, the average cost of PCSK9-inhibiting mAb therapy in the United States was $14,350 per year. Since then, the price of evolocumab and alirocumab has dropped by 60%, greatly increasing the affordability for high-risk patients. However, these treatments are usually necessary for the duration of a patient’s life, a factor that significantly contributes to their overall affordability. As such, the search for cheaper PCSK9 inhibitors and those with less frequent administration is always desirable. Inclisiran, a small interfering RNA (siRNA) which reduces LDL-C by 51% when given to patients on maximally tolerated statins, tackles the latter problem by requiring only 2-3 doses per year. However, it is a relatively new treatment and is currently more expensive than mAbs. A single dose of inclisiran costs $3250 in the U.S. Lastly, small molecules are more easily administered than mAbs or siRNA therapy, which both require a healthcare provider.

Experimental and Human Trials

In both human and animal experiments, berberine has been found to significantly reduce LDL-C through LDLR upregulation. Hyperlipidemic hamsters treated with 100mg/kg/day of berberine saw a 40% decrease in serum cholesterol and a 42% decrease in LDL-C compared to control animals on the same diet. Hepatic LDLR mRNA and protein levels of these hamsters were also significantly elevated (350% and 260%, respectively). Those given 50mg/kg/day of berberine for 10 days saw a 26% reduction in LDL-C. In a human study, 63 hypercholesterolemic participants received 500 mg of berberine twice daily for three months. LDL-C, triglycerides, and serum cholesterol levels decreased by 20%, 28%, and 18%, respectively, compared to placebo. Of the 32 participants on no other medications, the reductions were more drastic. LDL-C, triglycerides, and serum cholesterol were reduced by 29%, 35%, and 25%, respectively. A recent, double-blind study with 49 subjects found that 900 mg of berberine given daily for eight weeks significantly reduced total cholesterol, LDL-C, fasting serum insulin, and insulin resistance. In a 144-person study, 1 gram of berberine for three months produced reductions in total cholesterol (TC), LDL-C, and TG of 11.6%, 16.4%, and 21.2%, respectively, compared to placebo. Berberine reduces these markers in a time and dose-dependent manner.

Berberine is a potent reducer of major CVD risk factors. The combination of simvastatin with berberine confirms earlier research that statins and berberine work synergistically; together they produced a 31.8%, 38.9%, and 29.1% decrease in LDL-C, TG, and TC, respectively. Nutraceutical combinations of berberine with policosanol and red yeast rice have also been shown to be more effective at reducing total cholesterol, triglycerides, LDL-C, and non-HDL-C than berberine alone or ezetimibe, a cholesterol-lowering drug that is commonly prescribed with statins or to those who are statin intolerant. The main active ingredient in red yeast rice is monacolin K, which is structurally identical to lovastatin. This further corroborates the efficacy of combination therapy with berberine and statins. Although berberine is well-tolerated and has no reported significant side effects, long-term use may be accompanied by complications unseen to date. Low bioavailability and the lack of large-scale and long-term clinical trials are berberine’s current limitations.

Synthetic Berberine Derivatives

Several attempts have been made to improve berberine’s bioavailability. Different methods for synthesizing berberine derivatives include synthesis of non-natural derivatives and drug delivery nanotechnology. There are many berberine derivatives currently being investigated for their PCSK9 inhibiting and lipolowering properties. In rat trials, 8-hydroxydihydroberberine was found to decrease LDL-C by 73% and total cholesterol by about 60%. It also increases LDLR expression by 166 to 216%. A tetrahydroprotoberberine derivative named compound 22 decreased PCSK9 protein levels by 79% compared to the vehicle group (DMSO). In comparison, berberine decreased PCSK9 levels by 29%. Compound 22 also possessed an oral bioavailability of 21.9%. The methoxy group at the 12-position played a role in achieving a higher
bioavailability and lower PCSK9 expression. Adding an acetone molecule to berberine’s C-8 produced a derivative called berberine8998; its bioavailability is 6.7 times that of berberine. It also outperformed berberine in TC, TG, and LDL-C reduction in a trial using six-week-old hamsters on a high-fat diet. In another study, converting the 9-position methoxy group on berberine to alcohol increased bioavailability. This compound, 5g, was found to decrease LDL-C by 45.5% and cholesterol by 35.8% compared to controls in rats fed a high fat, high cholesterol diet for 28 days. In contrast, berberine reduced LDL-C by 38% and cholesterol by 27.4%. Compound 1m reduced LDL-C and TC by 41 and 20%, respectively, in rats. It performed better in HepG2 cells, where it decreased LDL-C by 70% and TC by 50%.

Another new berberine derivative, 9k, shows impressive PCSK9 inhibitory effects and LDLR upregulation. In HepG2 cells, it was found to reduce PCSK9 expression by 98%. LDLR expression increased by 90-300% in a dose-dependent manner. It was well tolerated in mice, with an LD50 > 1000 mg and increased LDL-C uptake by 60-150%. Interestingly, 9k reduces PCSK9 via HNF1α modulation, similar to berberine. Mutations in the HNF1α binding site abolished PCSK9 inhibition. Berberrubine, one of berberine’s phase I metabolites, also shows extensive bioactivity. It was shown to lower PCSK9 expression by 74% and TC by 37% in HepG2 cells. A manmade analog, hydroxypropyl-berberrubine, reduced PCSK9 expression and total cholesterol by 58% and 30%, respectively. Another compound, 2h-1, was found to reduce TC by >50% and TG by 70% in HepG2 cells compared to controls. 8-cetylberberine is another analog which lowered total cholesterol and LDL-C by 48% and 22%, respectively, in hyperlipidemic mice. While each of these compounds offer a distinct method for increasing efficacy and bioavailability, the majority of berberine analogs with improved bioavailability and potency show increased lipophilicity compared to berberine. There are also certain moieties that tend to confer more desirable traits, such as modification at the C-9 position and 9-O-cinnamic substitutions. Table 1 summarizes data on all available berberine derivatives that demonstrate hypolipidemic properties and/or PCSK9 inhibition.

### Table 1: Berberine Derivatives with Hypolipidemic Properties

<table>
<thead>
<tr>
<th>Compound</th>
<th>Stage</th>
<th>Bioavailability</th>
<th>PCSK9 Reduction</th>
<th>LDL-C Reduction</th>
<th>TC Reduction</th>
<th>LDLR Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Berberine</td>
<td>n/a</td>
<td>0.37%**24</td>
<td>29% to 87%***</td>
<td>16 to 4,89,104</td>
<td>18 to 42%***</td>
<td>260%##4</td>
</tr>
<tr>
<td>8-hydroxydihydroberberine*</td>
<td>Preclinical</td>
<td>Not Quantified</td>
<td>Up to 25%</td>
<td>73.7%</td>
<td>58.5 to 68.8%</td>
<td>166 to 216%</td>
</tr>
<tr>
<td>1m122</td>
<td>Preclinical</td>
<td>Not Quantified</td>
<td>Not</td>
<td>41.5%* to 70%**</td>
<td>19.6%* to 50%**</td>
<td>Quantified</td>
</tr>
<tr>
<td>2109</td>
<td>Preclinical</td>
<td>Not Quantified</td>
<td>Not</td>
<td>49.4%* to 42.6%*</td>
<td>Not Quantified</td>
<td>350%**</td>
</tr>
<tr>
<td>9k123</td>
<td>Preclinical</td>
<td>Not Quantified</td>
<td>38%# to 98%**</td>
<td>LDL-C uptake increased 60 to 150%**</td>
<td>Not Quantified</td>
<td>190 to 400%**</td>
</tr>
<tr>
<td>22#118 (racemate)</td>
<td>Preclinical</td>
<td>21.9%</td>
<td>79%</td>
<td>41.4%</td>
<td>36.7%</td>
<td>206%</td>
</tr>
<tr>
<td>5g (prodrug)121</td>
<td>Preclinical</td>
<td>Not Quantified</td>
<td>Not</td>
<td>45.5%</td>
<td>35.8%</td>
<td>No upregulation</td>
</tr>
<tr>
<td>8-cetylberberine#125</td>
<td>Preclinical</td>
<td>Not Quantified</td>
<td>Not</td>
<td>22.6%</td>
<td>47.9%</td>
<td>Not Quantified</td>
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<tr>
<td>Berberine8998119</td>
<td>Preclinical</td>
<td>1.3## to 2.5%*</td>
<td>Not</td>
<td>35 to 41.7%##</td>
<td>Not Quantified</td>
<td>Not Quantified</td>
</tr>
<tr>
<td>Berberrubine*92</td>
<td>Preclinical</td>
<td>Not Quantified</td>
<td>74%</td>
<td>Not Quantified</td>
<td>37%</td>
<td>135%</td>
</tr>
</tbody>
</table>
Table 1: Compound Stage Bioavailability PCSK9 Reduction LDL-C Reduction TC Reduction LDLR Expression

<table>
<thead>
<tr>
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<th>LDL-C Reduction</th>
<th>TC Reduction</th>
<th>LDLR Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxypropyl-berberrubrine**92</td>
<td>Preclinical</td>
<td>Not Quantified</td>
<td>58%</td>
<td>Not Quantified</td>
<td>30.4%</td>
<td>160%</td>
</tr>
<tr>
<td>2H-1***124</td>
<td>Preclinical</td>
<td>Not Quantified</td>
<td>Not</td>
<td>Not</td>
<td>&gt;50%</td>
<td>Not Quantified</td>
</tr>
</tbody>
</table>

Table 1 organizes data about emerging berberine derivatives which show promising results in lowering LDL-C and total cholesterol. Many of these compounds also inhibit PCSK9 and increase LDLR expression in similar ways to berberine. All compounds were either well tolerated or produced minimal cytotoxicity (<10%). [# - hyperlipidemic mice trials,## - high fat diet hyperlipidemic hamster trials,* - rat trials, ** - in HepG2 or Huh7 cells, *** - human trials]

Conclusion

Diabetes, CVD, cancer, and Alzheimer’s disease share a common pathology of chronic inflammation. Berberine has been shown to attenuate these inflammatory pathways by reducing the expression of the inflammatory cytokines TLR4, IL-1β, IL-1, and TNF-α, which are elevated in all three diseases. TLR4 is activated by NF-κB, which berberine is known to decrease. Berberine also decreases caspase-1 protein levels; caspase-1 creates IL-1β upon self-activation. Berberine halts expression of cyclooxygenase-2 (COX-2) and prostaglandin E₂. COX-2 is activated during inflammation, wound healing, and metabolic disorders. It is the main enzyme in the production of prostaglandins and produces prostaglandin H₂ (PGH₂). Prostaglandin E₂ synthase converts PGH₂ into prostaglandin E₂, which can stimulate cancer progression. Prostaglandins have been found to interact with cytokines by inducing cytokine receptor expression and via upregulation of inflammatory genes such as COX-2 and NF-κB. Feedback between prostaglandins and cytokines creates a cycle of escalating inflammation. All of these mechanisms contribute to berberine’s anti-inflammatory properties.

By reducing inflammation, berberine reduces amyloid beta accumulation and tau hyperphosphorylation in mice and various cell lines. Berberine is yet to reach human trials in Alzheimer’s research. It enhances insulin sensitivity and secretion by pancreatic β-cells in mice and several small, double-blind clinical trials. Clinical trials in diabetes patients show improved glycemic and lipid profiles. Berberine also improves CVD outcomes. In one study, 130 patients with acute coronary syndrome who underwent percutaneous coronary intervention (PCI) were given either 900 mg of berberine plus standard therapy or standard therapy alone for 30 days. The berberine group saw a reduction in inflammation caused by PCI, measured as a reduction in matrix metalloproteinase (MMP)-9, intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, C-reactive protein, interleukin-6 and monocyte chemoattractant protein-1 levels.

There are a few clinical trials detailing berberine’s effects on cancer, mostly involving women with polycystic ovary syndrome. In addition, berberine reduced radiation-induced lung injury in non-small cell lung cancer patients. In a separate study, it reduced the risk of recurrence of colorectal adenoma.

Berberine kills cancer cells in vitro and also improves doxorubicin resistance and related myocardial injury in cancer patients. While results in cell lines and rodent models are promising, more large-scale clinical trials with berberine are warranted. Cancer, T2DM, and AD have complicated pathologies which cannot be fully and accurately represented by model systems.

Berberine is a naturally occurring PCSK9 inhibitor that also upregulates and stabilizes LDLR mRNA. Berberine inhibits PCSK9 transcription via degradation of the HNF1α protein, one of two transcription factors required for PCSK9 transcription. PCSK9 is crucial in LDL-C metabolism due to its interaction with LDL receptors in the liver; clinically, PCSK9 inhibition leads to significant LDL-C reductions in both human trials and animal models. Berberine reduces PCSK9 mRNA and protein levels with...
This increases the amount of LDL receptors, as fewer are degraded due to PCSK9-LDLR interactions. An increase in LDL receptors increases LDL-C plasma clearance, resulting in the beneficial anti-atherosclerotic effects of berberine. Berberine’s stabilization of LDLR mRNA also contributes to these effects, as does its ability to reduce intestinal cholesterol absorption and excrete excess cholesterol from the liver into the bile. It is clinically proven to reduce LDL cholesterol by 20%, triglycerides by 20-30%, and has been well-tolerated with no reported major side effects. Berberine’s low bioavailability is its major drawback; however, this issue is currently being addressed successfully by numerous researchers. Berberine derivatives have emerged with impressively augmented bioavailabilities and increased LDL-C reductions. As synthetic berberine derivatives continue through clinical trials, we may very well see a new class of anti-cardiovascular disease drugs emerge on the market.

Berberine has the potential to become a key compound in the fight against atherosclerosis, diabetes, AD, and other age-related illnesses. Neuroprotection and cognitive improvement are two areas of great potential for future development, especially considering that age-related illnesses will continue to rise throughout the century. AD currently affects over 6 million adults in the U.S. That number is expected to double by 2060 if effective treatments are not found. Cardiovascular disease is the leading cause of death worldwide. Diabetes is the 8th, affecting 37 million people in the United States. A drug with the potential to treat all three could revolutionize the medical field.

References


(86) Lona, J. M. F.; Martínez, M. S.; Alarcón, G. V.; Rodas, A. B. El factor de necrosis tumoral α (TNF-α) en las enfermedades cardiovasculares: biología molecular y genética. *Gac. Médica México*.


(115) Efficacy and Tolerability of a Nutraceutical Combination (Red Yeast Rice, Policosanols, and Berberine) in Patients with Low-Moderate Risk Hypercholesterolemia: A Double-Blind, Placebo-Controlled Study - ClinicalKey. https://www-clinicalkey-com.ezproxy3.library.arizona.edu/#!/content/playContent/1-s2.0-S0011393X14000162?returnurl=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS0011393X14000162%3Fshowall%3Dtrue&referrer=


(120) Wang, Y.-X.; Kong, W.-J.; Li, Y.-H.; Tang, S.; Li, Z.; Li, Y.-B.; Shan, Y.-Q.; Bi, C.-W.; Jiang, J.-D.; Song, D.-Q. Synthesis and Structure–Activity Relationship of Berberine Analogues


