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Atorvastatin and ezetimibe protect against hypercholesterolemia: An overview

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Abstract
High blood levels of triglycerides (fats) and cholesterol are treated with a combination of atorvastatin and ezetimibe in conjunction with a balanced diet. This medication may aid in the prevention of illnesses (such as heart attacks and strokes) brought on by blocked blood arteries. HMG-CoA reductase inhibitors, or statins, include atorvastatin and ezetimibe, which both limit the absorption of cholesterol. Both the amount of cholesterol your body produces and absorbs from food will be decreased by these medications. This article explains the various analytical techniques that have been developed and validated in accordance with ICH guidelines for the determination of atorvastatin and ezetimibe. This review describes the drugs like atorvastatin and ezetimibe description, pharmacodynamics, pharmacokinetics, and toxicity. These techniques include UV-Spectrophotometry, Ultra Performance Liquid Chromatography (UPLC), Mass spectrometric, Liquid chromatography-Mass spectroscopy (LC-MS). It is crucial to analyze the drug content and purity percentage in bulk and pharmaceutical formulations for quality control purposes.

Keywords: Cholesterol, UV-spectrophotometric methods, HMG-CoA reductase, hypercholesterolemia

Introduction

Atorvastatin is an HMG-CoA reductase inhibitor used to lower lipid levels and reduce the risk of cardiovascular disease including myocardial infarction and stroke. Atorvastatin IUPAC name is [(3R, 5R)-7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-propan-2-ylpyrrol1-yl]-3,5-dihydroxy heptanoic acid].

Atorvastatin, is a lipid-lowering drug included in the statin class of medications. By inhibiting the endogenous production of cholesterol in the liver, statins lower abnormal cholesterol and lipid levels, and ultimately reduce the risk of cardiovascular disease. More specifically, statin medications competitively inhibit the enzyme hydroxy methyl glutaryl-coenzyme A (HMG-CoA) Reductase, which catalyzes the conversion of HMG-CoA to mevalonic acid. This conversion is a critical metabolic reaction involved in the production of several compounds involved in lipid metabolism and transport, including cholesterol, low-density lipoprotein (LDL) (sometimes referred to as "bad cholesterol"), and very-low-density lipoprotein (VLDL). Prescribing statins is considered standard practice for patients following any cardiovascular event, and for people who are at moderate to high risk of developing cardiovascular disease [1-4].

Atorvastatin was first synthesized in 1985 by Dr. Bruce Roth and approved by the FDA in 1996. It is a penta-substituted pyrrole formed by two contrasting moieties with an achiral heterocyclic core unit and a 3,5-dihydroxy pentanoyl side chain identical to its parent compound. Unlike other members of the statin group, atorvastatin is an active compound and therefore does not require activation [5-7].

Pharmacodynamics

Atorvastatin is an oral antilipemic agent that reversibly inhibits HMG-CoA reductase. It lowers total cholesterol, low-density lipoprotein-cholesterol (LDL-C), apolipoprotein B (apo B), non-high-density lipoprotein-cholesterol (non-HDL-C), and triglyceride (TG) plasma concentrations while increasing HDL-C concentrations. High LDL-C, low HDL-C and high TG concentrations in the plasma are associated with increased risk of atherosclerosis and cardiovascular disease. The total cholesterol to HDL-C ratio is a strong predictor of coronary artery disease, and high ratios are associated with a higher risk of disease. Increased levels of HDL-C are associated with lower cardiovascular risk. By decreasing LDL-C and TG and increasing HDL-C, atorvastatin reduces the risk of cardiovascular morbidity and mortality.

Elevated cholesterol levels (and high low-density lipoprotein (LDL) levels in particular) are an important risk factor for the development of CVD. Clinical studies have shown that atorvastatin reduces LDL-C and total cholesterol by 36-53%. In patients with dysbetalipoproteinemia, atorvastatin reduced the levels of intermediate-density lipoprotein cholesterol. It has also been suggested that atorvastatin can limit the extent of angiogenesis, which can be useful in the treatment of chronic subdural hematoma [8-11].

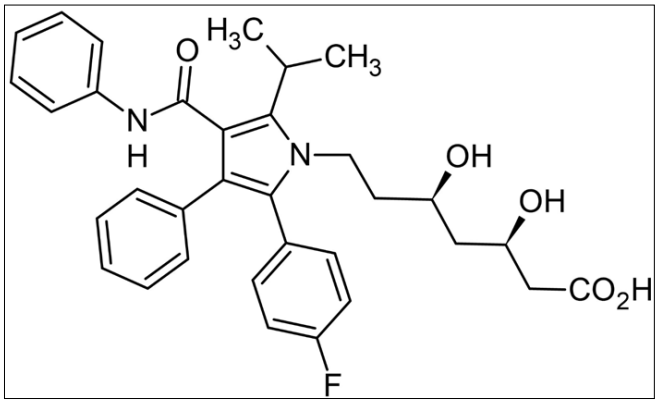

Fig 1: Structure of atorvastatin

Mechanism of action

Atorvastatin is a statin medication and a competitive inhibitor of the enzyme HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase, which catalyzes the conversion of HMG-CoA to mevalonate, an early rate-limiting step in cholesterol biosynthesis. Atorvastatin acts primarily in the liver, where decreased hepatic cholesterol concentrations stimulate the upregulation of hepatic low-density lipoprotein (LDL) receptors, which increases hepatic uptake of LDL. Atorvastatin also reduces Very-Low-Density Lipoprotein-Cholesterol (VLDL-C), serum triglycerides (TG) and Intermediate Density Lipoproteins (IDL), as well as the number of apolipoprotein B (apo B) containing particles, but increases High-Density Lipoprotein Cholesterol (HDL-C).

In vitro and *in vivo* animal studies also demonstrate that atorvastatin exerts vasculo-protective effects independent of its lipid-lowering properties, also known as the pleiotropic effects of statins. These effects include improvement in endothelial function, enhanced stability of atherosclerotic plaques, reduced oxidative stress and inflammation, and inhibition of the thrombogenic response. Statins were also found to bind allosterically to β2 integrin function-associated antigen-1 (LFA-1), which plays an essential role in leukocyte trafficking and T cell activation [3, 12].

Absorption

Atorvastatin presents a dose-dependent and non-linear pharmacokinetic profile. It is very rapidly absorbed after oral administration. After the administration of a dose of 40 mg, its peak plasma concentration of 28 ng/ml is reached 1-2 h after initial administration with an AUC of about 200 ng·h/ml. Atorvastatin undergoes extensive first-pass metabolism in the wall of the gut and the liver, resulting in an absolute oral bioavailability of 14%. Plasma atorvastatin concentrations are lower (approximately 30% for Cmax and AUC) following evening drug administration compared with morning. However, LDL-C reduction is the same regardless of the time of day of drug administration.

Metabolism

Atorvastatin is highly metabolized to ortho- and para-hydroxylated derivatives and various beta-oxidation products, primarily by Cytochrome P450 3A4 in the intestine and liver. Atorvastatin's metabolites undergo further lactonization via the formation of acyl glucuronide intermediates by the enzymes UGT1A1 and UGT1A3. These lactones can be hydrolyzed back to their corresponding acid forms and exist in equilibrium. *In vitro* inhibition of HMG-CoA reductase by ortho- and para-hydroxylated metabolites is equivalent to that of atorvastatin. Approximately 70% of circulating inhibitory activity for HMG-CoA reductase is attributed to active metabolites.

Route of elimination

Atorvastatin and its metabolites are mainly eliminated in the bile without enterohepatic recirculation. The renal elimination of atorvastatin is very minimal and represents less than 1% of the eliminated dose

Half-life

The half-life of atorvastatin is 14 h while the half-life of its metabolites can reach up to 30 h.

Protein binding

Atorvastatin is highly bound to plasma proteins and over 98% of the administered dose is found in a bound form.

Clearence

The registered total plasma clearance of atorvastatin is of 625 ml/min.

Toxicity

The reported LD50 of oral atorvastatin in mice is higher than 5000 mg/kg. In cases of overdose with atorvastatin, there is reported symptoms of complicated breathing, jaundice, liver damage, dark urine, muscle pain, and seizures. In case of overdose, symptomatic treatment is recommended and due to the high plasma protein binding, hemodialysis is not expected to generate significant improvement [13-15].

Ezetimibe is a cholesterol absorption inhibitor used to lower total cholesterol, LDL-C, Apo-B, and non-HDL-C in primary hyperlipidemia and familial cholesterolemia. Ezetimibe IUPAC name is [(3*R*,4*S*)-1-(4-fluorophenyl)-3-[(3*S*)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)-2-azetidinone].

Back ground

Ezetimibe is a lipid-lowering compound that inhibits intestinal cholesterol and phytosterol absorption. The discovery and research of this drug began in the early 1990s, after the intravenous administration of radiolabelled ezetimibe in rats revealed that it was being localized within enterocytes of the intestinal villi - this prompted studies investigating the effect of ezetimibe on intestinal cholesterol absorption. Ezetimibe is used as an adjunctive therapy to a healthy diet to lower cholesterol levels in primary hyperlipidemia, mixed hyperlipidemia, homozygous familial hypercholesterolemia (HoFH), and homozygous sitosterolemia (phytosterolemia).

Pharmacodynamics

Ezetimibe was shown to reduce the levels of total cholesterol (total-C), low-density lipoprotein cholesterol (LDL-C), apoprotein B (Apo B), non-high-density lipoprotein cholesterol (non-HDL-C), and triglycerides (TG), and increase high-density lipoprotein cholesterol (HDL-C) in patients with hyperlipidemia. This therapeutic effect was more profound when ezetimibe was co-administered with a statin or fenofibrate compared to either treatment alone. In clinical trials involving patients with homozygous and heterozygous familial hypercholesterolemia and in those with sitosterolemia, a recommended therapeutic dose of ezetimibe was effective in reducing the LDL levels by 15-20% while increasing HDL-C by 2.5-5%.

The effects of increased exposure to ezetimibe secondary to moderate-severe hepatic impairment have not been assessed - patients meeting these criteria should avoid the use of ezetimibe. Post-marketing reports indicate the potential for myopathy and rhabdomyolysis in patients taking ezetimibe, and this risk appears to be exacerbated in patients concurrently receiving, or having recently received, statin therapy [16-18].

Mechanism of action

Ezetimibe mediates its blood cholesterol-lowering effect via selectively inhibiting the absorption of cholesterol and phytosterol by the small intestine without altering the absorption of fat-soluble vitamins and nutrients. The primary target of ezetimibe is the cholesterol transport protein Niemann-Pick C1-Like 1 (NPC1L1) protein. NPC1L1 is expressed on enterocytes/gut lumen (apical) as well as the hepatobiliary (canalicular) interface and plays a role in facilitating internalization of free cholesterol into the enterocyte in conjunction with the adaptor protein 2 (AP2) complex and clathrin. Once cholesterol in the gut lumen or bile is incorporated into the cell membrane of enterocytes, it binds to the sterol-sensing domain of NPC1L1 and forms a NPC1L1/cholesterol complex. The complex is then internalized or endocytosed by joining to AP2 clathrin, forming a vesicle complex that is translocated for storage in the endocytic recycling compartment.

Ezetimibe does not require exocrine pancreatic function for its pharmacological activity; rather, it localizes and appears to act at the brush border of the small intestine. Ezetimibe selectively blocks the NPC1L1 protein in the jejunal brush border, reducing the uptake of intestinal lumen micelles into the enterocyte. Overall, ezetimibe causes a decrease in the delivery of intestinal cholesterol to the liver and reduction of hepatic cholesterol stores and an increase in clearance of cholesterol from the blood. While the full mechanism of action of ezetimibe in reducing the entry of cholesterol into both enterocytes and hepatocytes is not fully understood, one study proposed that ezetimibe prevents the NPC1L1/sterol complex from interacting with AP2 in clathrin coated vesicles and induces a conformational change in NPC1L1, rendering it incapable of binding to sterols. Another study suggested that ezetimibe disrupts the function of other protein complexes involved in regulating cholesterol uptake, including the CAV1–annexin 2 heterocomplex.

Metabolism

In humans, ezetimibe is rapidly and extensively metabolized via a phase II glucuronide conjugation reaction in the small intestine and liver to form its main phenolic metabolite, ezetimibe glucuronide. The main human liver and/or intestinal uridine 5′-diphosphate (UDP)-glucuronosyltransferase (UGT) enzymes responsible for the glucuronidation of ezetimibe were shown to be UGT1A1, 1A3, and 2B15 *in vitro*. Minimal phase I reaction involving oxidation of ezetimibe also occurs to form SCH 57871, and human jejunum microsomes also produced trace levels of a benzylic glucuronide (SCH 488128). Ezetimibe glucuronide accounts for 80-90% of the total circulating compound in plasma, and retains some pharmacological activity in inhibiting intestinal cholesterol uptake. In humans, ezetimibe and ezetimibe-glucuronide constitutes approximately 93% of the total drug in plasma. Plasma concentration-time profiles exhibit multiple peaks, suggestive of enterohepatic recycling, and about 20% of the drug distributed is reabsorbed due to enterohepatic recirculation.

Half-life

Both ezetimibe and ezetimibe-glucuronide display an approximate half-life of 22 h.

Toxicity

Oral LD_{50} and intraperitoneal LD_{50} in rat were >2000 mg/kg. Estimated oral LD50 values in mouse and dog are >5000 mg/kg and >3000 mg/kg, respectively. One case of accidental overdose occurred in clinical studies in one female patient with homozygous sitosterolemia receiving 120 mg/day for 28 days with no reported clinical or laboratory adverse events. In case of overdose, symptomatic treatment is recommended.

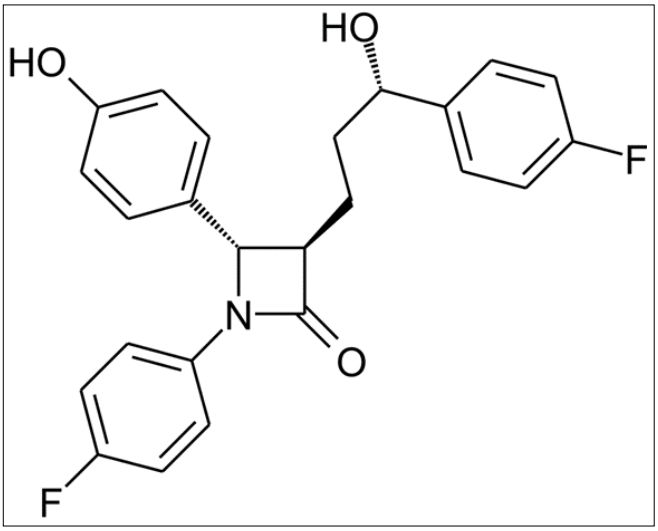

Fig 2: Structure of ezetimibe

Route of elimination

Approximately 78% and 11% of orally administered radiolabelled ezetimibe are recovered in the feces and urine, respectively.[5] Unchanged parent drug is the major component in feces and accounts for approximately 69% of an administered dose, while ezetimibe-glucuronide is the major component in urine and accounts for approximately 9% of an administered dose.[5] High recovery of unchanged parent drug in feces suggests low absorption and/or hydrolysis of ezetimibe-glucuronide secreted in the bile.

Absorption

Administration of a single 10-mg dose of ezetimibe in fasted adults resulted in peak plasma concentrations (C_{max}) of 3.4-5.5 ng/mL within 4-12 h (T_{max}).[5] The C_{max} of the major pharmacologically-active metabolite, ezetimibe-glucuronide, was 45-71 ng/mL and its T_{max} was 1-2 h. Food consumption has minimal effect on ezetimibe absorption, but the C_{max} is increased by 38% when administered alongside a high-fat meal.[5] The true bioavailability of ezetimibe cannot be determined, as it is insoluble in aqueous media suitable for intravenous injection.

Protein binding

Ezetimibe and ezetimibe-glucuronide are >90% bound to human plasma proteins. The mean *in vitro* protein binding ranged from 99.5% to 99.8% for ezetimibe and 87.8% to 92.0% for ezetimibe-glucuronide [18-20].

Various analytical methods

The various developed analytical methods which are used for the estimation of atorvastatin and ezetimibe in combination are explained. Developed analytical methods involve UV-spectroscopy, HPTLC (High-Performance Thin

Layer Chromatography), UPLC (Ultra Performance Liquid Chromatography), and some other methods [21, 22].

Table 1: Analytical methods developed

S. No.	Author name, year	Method done
1	Nada S, 2012	simple, accurate, precise and economic spectrophotometric methods have been developed for simultaneous determination of Atorvastatin calcium (ATR) and Ezetimibe (EZ) in their bulk powder and pharmaceutical dosage form. Method (I) is based on dual wavelength analysis while method (II) is the mean centering of ratio spectra spectrophotometric (MCR) method. In method (I), two wavelengths were selected for each drug in such a way that the difference in absorbance was zero for the second drug. At wavelengths 226.6 and 244 nm EZ had equal absorbance values; therefore, these two wavelengths have been used to determine ATR; on a similar basis 228.6 and 262.8 nm were selected to determine EZ in their binary mixtures.
2	Shravya A, 2010	simple sensitive and cost-effective spectrophotometric methods are described for the determination of atorvastatin calcium and ezetimibe in bulk and pharmaceutical formulations. The method is based on the oxidation of 2,4-dinitrophenylhydrazine and coupling of the oxidized product with drugs to give intensely colored chromogen.
3	Tarek S, 2013	new simple spectrophotometric method was developed for the determination of binary mixtures without prior separation. The method is based on the generation of ratio spectra of compound *X* by using a standard spectrum of compound *Y* as a divisor. The peak to trough amplitudes between two selected wavelengths in the ratio spectra are proportional to concentration of *X* without interference from *Y*. The method was demonstrated by determination of two drug combinations. The first consists of the two antihyperlipidemics: atorvastatin calcium (ATV) and ezetimibe (EZE), and the second comprises the antihypertensives: candesartan cilexetil (CAN) and hydrochlorothiazide (HCT).
4	Yehia Z, 2013	Three sensitive methods were developed for simultaneous determination of Ezetimibe (EZB) and Atorvastatin calcium (ATVC) in binary mixtures. First derivative (D1) spectrophotometry was employed for simultaneous determination of EZB (223.8 nm) and ATVC (233.0 nm).
5	Deshmukh DD, 2008	simple, sensitive and rapid colorimetric method for estimation of ezetimibe and spectrophotometric method for simultaneous estimation of atorvastatin calcium and ezetimibe in tablet formulations have been developed. Colorimetric method for the estimation of ezetimibe was based on the formation of ion pair complex of drug with dye. The method was based on the formation of bluish-green coloured complex with patent blue-V and hydrochloric acid. The coloured complex showed absorbance maxima at 636 nm and obeyed Beer's law in the concentration range of 20-50 µg/mL.
6	Ramzia I, 2013	LC–MS–MS method has been developed and validated for the simultaneous estimation of atorvastatin and ezetimibe in human plasma using pitavastatin as an internal standard. Liquid–liquid extraction was used for the purification and preconcentration of analytes from human plasma matrix. The chromatographic separation was achieved within 3.0 min by an isocratic mobile phase consisting of 0.2% formic acid in water–acetonitrile (30:70, v/v), flowing through Agilent Eclipse-plus C18, 100 × 4.6 mm, 3.5 µm analytical column, at a flow rate of 0.6 mL min–1.
7	Blanka Szekely-Szentmiklosi, 2017	rapid and sensitive micellar electrokinetic capillary chromatography method with UV photodiode array detection was developed for the simultaneous determination of atorvastatin and ezetimibe in fixed dose drug combination. Experimental conditions such as buffer concentration and pH, surfactant concentration, system temperature, applied voltage, injection parameters were optimized in order to improve the efficiency of the separation. The best results were obtained when using fused silica capillary (48 cm length X 50 µm ID) and 25 mM borate buffer electrolyte at pH 9.3 containing 25 mM SDS, +30 kV applied voltage, 20 ^{0}C system temperature. The separation was achieved in approximately 2 min, with a resolution of 7.02, the order of migration being atorvastatin followed by ezetimibe.

Conclusion

Atorvastatin and ezetimibe may be used alone or combine with other cholesterol-lowering drugs to decreases in hepatic cholesterol storage and an increase in the clearance of cholesterol from the blood. Based on the results, it is concluded several UV spectroscopic and LC-MS methods were successfully developed for simultaneous estimations of atorvastatin and ezetimibe in bilk and pharmaceutical formulation. These methods can be useful for the routine analysis of atorvastatin and ezetimibe in combined dosage form.

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