Streptozotocin, Atorvastatin, Renal, Diabetes and Related Factors

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Abstract: Renal lipid metabolism may play important roles in renal inflammation, glomerulosclerosis and tubulointerstitial injury in diabetic nephropathy. The therapeutic effect of atorvastatin in streptozotocin (STZ)-induced diabetic rat and its impact on renal SREBP expression were studied for a long time. Streptozotocin (Streptozocin, STZ) is a chemical that is toxic to the insulin-producing beta cells of the pancreas in mammals. It is used for treating certain cancers of the Islets of Langerhans and also can be used in scientific research to produce an animal model for Type 1 diabetes in large dose as well as Type 2 diabetes with multiple low doses. Since it has a risk of toxicity, its use is generally limited to patients whose cancer cannot be removed by surgery. Atorvastatin (Lipitor), produced by Pfizer company as a calcium salt, is a member of the drug of statins used primarily for lowering blood cholesterol and for prevention of events associated with cardiovascular disease. Atorvastatin works by inhibiting HMG-CoA reductase, an enzyme in liver that plays a key role in production of cholesterol in the body. Atorvastatin causes a more dramatic reduction in LDL-C than the other statin drugs.
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Key words: atreptozotocin; atorvastatin (Lipitor); renal, diabetes; lipid

1. Introduction

The kidneys serve several essential regulatory roles in animals to remove excess organic molecules (waste products of metabolism) from the blood. They are essential in the urinary system and also serve homeostatic functions such as the regulation of electrolytes, maintenance of acid–base balance, and regulation of blood pressure. Kidneys are the filter of the blood to remove water soluble wastes to the urinary bladder, and they are also work to reabsorpt water, glucose, and amino acids, etc. The kidneys also produce hormones such as calcitriol, erythropoietin, and the enzyme renin, etc.

Diabetes is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period. This high blood sugar produces the symptoms of frequent urination, increased thirst, and increased hunger. Diabetes is due to either the pancreas not producing enough insulin, or the cells of the body not responding properly to the insulin produced. There are three main types of diabetes mellitus: (1) Type 1 diabetes results from the body's failure to produce enough insulin. (2) Type 2 diabetes begins with insulin resistance, a condition in which cells fail to respond to insulin properly. (3) Gestational diabetes is the third main form and occurs when pregnant women without a previous history of diabetes develop a high blood glucose level. Diabetic kidney disease is a complication that can progress to kidney failure.

Streptozotocin (Streptozocin, STZ) is a chemical that is toxic to the insulin-producing beta cells of the pancreas in mammals. It is used for treating certain cancers of the Islets of Langerhans and also can be used in scientific research to produce an animal model for Type 1 diabetes in large dose as well as Type 2 diabetes with multiple low doses. Since it has a risk of toxicity, its use is generally limited to patients whose cancer cannot be removed by surgery. In these patients, streptozotocin can reduce the tumor size and reduce symptoms (especially hypoglycemia due to excessive insulin secretion by insulinomas). Generally a dose of 500 mg/m^2/day by intravenous injection, for 5 days, repeated every 4-6 weeks could be applied.

Atorvastatin (Lipitor), produced by Pfizer company as a calcium salt, is a member of the drug of statins used primarily for lowering blood cholesterol and for prevention of events associated with cardiovascular disease. Atorvastatin works by inhibiting HMG-CoA reductase, an enzyme in liver that plays a key role in production of cholesterol in the body. Atorvastatin causes a more dramatic reduction in LDL-C than the other statin drugs. From 1996 to 2012 under the trade name Lipitor, atorvastatin became the world's best-selling drug of all time, with more than $125 billion in sales over approximately 14.5 years. When Pfizer's patent on Lipitor expired on 30 November 2011, generic atorvastatin became available in the United States. Initially, atorvastatin was manufactured only by generic drugmakers Watson Pharmaceuticals and India's Ranbaxy Laboratories.

The chemical structure of streptozotocin and atorvastatin are shown in Figure 1 and Figure 2 (Wikipedia, 2014).

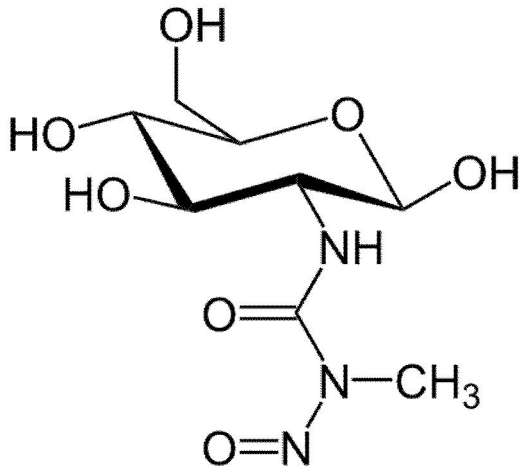

Figure 1. Streptozocin chemical structure (Yikrazuul, http://en.wikipedia.org/wiki/Streptozotocin, http://en.wikipedia.org/wiki/File:Streptozocin.svg, 2014)

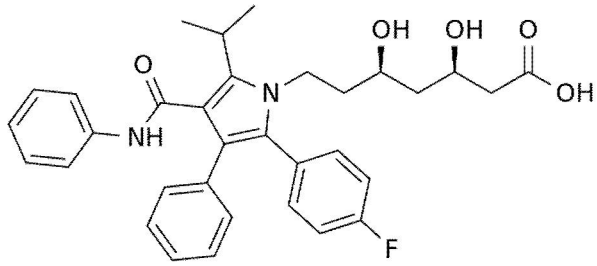

Figure 2. Atorvastatin chemical structure (http://en.wikipedia.org/wiki/Atorvastatin, http://en.wikipedia.org/wiki/File:Atorvastatin2DCSD.svg, 2014)

LIPITOR is a prescription medicine called a statin that lowers cholesterol in your blood. It lowers the LDL-C ("bad" cholesterol) and triglycerides in your blood. It can raise your HDL-C ("good" cholesterol) as well (http://www.lipitor.com).

Diabetes mellitus is the leading cause of cardiovascular and renal disease, and it is closed related to the lipid condition of the animal body. The pathogenesis of diabetic nephropathy is multi-factorial. Hypertension, hyperglycemia, profibrotic growth factors, including angiotensin II, transforming growth factor (TGF) and vascular endothelial growth factor (VEGF), proinflammatory cytokines, oxidative stress, and advanced glycation end products (AGEs) have been determined to play important roles in the pathogenesis of diabetic nephropathy (Bohlender et al., 2005; Brownlee, 2005; Chen et al., 2005; Cohen et al., 2005; Goh and Cooper, 2008; Nicholas et al., 2005; Wellen and Hotamisligil, 2005; Wendt et al., 2003; Yamagishi et al., 2007). In addition, abnormal lipid metabolism and renal accumulation of lipids have also been proposed to play a similar important role in the pathogenesis of diabetic nephropathy (Jiang et al., 2005a; Jiang et al., 2005b; Moorhead et al., 1982; Sun et al., 2002; Ma, et al, 2009).

Several studies in human subjects and in experimental animals with diabetes have shown a correlation between serum lipids, renal lipids, and proteinuria and progressive decline in renal function (Bonnet and Cooper, 2000; Spencer et al., 2004). Renal lipid accumulation mediated by increased renal lipid synthesis is involved in the nephropathy seen in animal models of type I diabetes, diet induced obesity and insulin resistance, and aging (Jiang et al., 2005a; Jiang et al., 2005b; Sun et al., 2002).

The purposes of the present study were to determine if there is a primary alteration in renal lipid metabolism, serum glucose and urine glucose in diabetes rats, and if the alterations are related to HMG-CoA, sterol response element binding protein-1 (SREBP-1), sterol response element binding protein-2 (SREBP-2), TNF-α, TGF-β1, TGF-β2, plasminogen activator inhibitor-1 (PAI-1), nephrin, podocin, ABCA1, α-actin, PPAR, VEGF, COX-2, and HIF expressions, and the histopathological consequences of diabetes.

Diabetic renal disease is associated with lipid deposits in the kidney (Lim et al., 2005; Sun et al., 2002). TNF-α, TGF-β1, TGF-β2, plasminogen activator inhibitor-1 (PAI-1), nephrin, podocin, ABCA1, α-actin, PPAR, VEGF, COX-2, and HIF expressions, and the histopathological consequences of diabetes.

In 2002, Sun et al reported the relationship of diabetic renal disease and sterol regulatory element-binding proteins (SREBPs): The purpose of their study was to determine whether there is altered regulation of the SREBPs in the diabetic kidney and whether SREBPs mediate the abnormal renal lipid metabolism and diabetic renal disease. In streptozotocin-induced diabetes in the rat, there are marked increases in SREBP-1 and fatty acid synthase (FAS) expression, resulting in increased triglyceride (TG) accumulation. Treatment of diabetic rats with insulin prevented the increased renal expression of SREBP-1 and the accumulation of TG. The role of hyperglycemia in the up-regulation of SREBP-1 was confirmed in renal cells cultured in a high glucose media. High glucose induced increased expression of SREBP-1a and -1c mRNA, SREBP-1 protein, and FAS, resulting in increased TG content. To determine a direct role for SREBP in mediating the increase in renal lipids and glomerulosclerosis, they studied SREBP-1a transgenic mice with increased renal

expression of SREBP-1. The increase in SREBP-1 was associated with increased expression of FAS and acetyl CoA carboxylase, resulting in increased TG content, increased expression of transforming growth factor beta1 (TGF-β1) and vascular endothelial growth factor (VEGF), mesangial expansion, glomerulosclerosis, and proteinuria. Their study therefore indicated that renal SREBP-1 expression was increased in diabetes and that SREBP-1 played an important role in the increased lipid synthesis, TG accumulation, mesangial expansion, glomerulosclerosis, and proteinuria by increasing the expression of TGF and VEGF (Lim et al., 2005; Sun et al., 2002).

It was reported that in type 1 diabetes, there is altered renal lipid metabolism favoring net accumulation of triglycerides and cholesterol, which are driven by increases in SREBP-1, ChREBP, and SREBP-2 and decreases in FXR, LXR-alpha, and LXR-beta, which may also play a role in the increased expression of profibrotic growth hormones, proinflammatory cytokines, and oxidative stress (Proctor et al., 2006). FXR agonists modulate renal SREBP-1 expression and lipid metabolism and renal expression of profibrotic growth factors, proinflammatory cytokines, and oxidative stress enzymes and decrease glomerulosclerosis, tubulointerstitial fibrosis, and proteinuria (Jiang et al., 2007). In FVBdb/db mice, renal triglyceride and cholesterol accumulation is mediated by increased activity of SREBP-1 and -2 (Wang et al., 2005).

Transforming growth factor alpha (TGF-α) is upregulated in some human cancers. It is produced in macrophages, brain cells, and keratinocytes, and induces epithelial development. It is closely related to EGF, and can also bind to the EGF receptor with similar effects. TGFα stimulates neural cell proliferation in the adult injured brain (Fallon et al., 2000). TGFα was cited in the 2001 NIH Stem Cell report to the U.S. Congress as promising evidence for the ability of adult stem cells to restore function in neurodegenerative disorders.

TGF-β acts synergistically with TGF-α in inducing cellular transformation. Specific receptors for TGF-β activation trigger apoptosis when activated. Many cells synthesize TGF-β and almost all of them have specific receptors for this peptide. TGF-β1, TGF-β2, and TGF-β3 all function through the same receptor signaling systems.

The peptide structures of the three members of the TGF-β family are highly similar. They are all encoded as large protein precursors; TGF-β1 contains 390 amino acids and TGF-β2 and TGF-β3 each contain 412 amino acids. They each have an N-terminal signal peptide of 20-30 amino acids that they require for secretion from a cell, a pro-region (called latency associated peptide, and a 112-114 amino acid C-terminal region that becomes the mature TGF-β molecule following its release from the pro-region by proteolytic cleavage. The mature TGF-β protein dimerizes to produce a 25 KDa active molecule with many conserved structural motifs. TGF-β has nine cysteine residues that are conserved among its family; eight form disulfide bonds within the molecule to create a cysteine knot structure characteristic of the TGF-β superfamily while the ninth cysteine forms a bond with the ninth cysteine of another TGF-β molecule to produce the dimer. Many other conserved residues in TGF-β are thought to form secondary structure through hydrophobic interactions. The region between the fifth and sixth conserved cysteines houses the most divergent area of TGF-β molecules that is exposed at the surface of the molecule and is implicated in receptor binding and specificity of TGF-β.

TGF-β induces apoptosis in numerous cell types. TGF-β can induce apoptosis in two ways: The SMAD pathway or the DAXX pathway. The SMAD pathway is the classical signaling pathway that TGF-β family members signal through. In this pathway, TGF-β dimers binds to a type II receptor which recruits and phosphorylates a type I receptor. The type I receptor then recruits and phosphorylates a receptor regulated SMAD (R-SMAD). SMAD3, an R-SMAD, has been implicated in inducing apoptosis. The R-SMAD then binds to the common SMAD (coSMAD) SMAD4 and forms a heterodimeric complex. This complex then enters the cell nucleus where it acts as a transcription factor for various genes, including those to activate the mitogen-activated protein kinase 8 pathway, which triggers apoptosis.

TGF-β may also trigger apoptosis via the death associated protein 6 (DAXX adapter protein). DAXX has been shown to associate with and bind to the type II TGF-β receptor kinase. TGF-β plays a crucial role in the regulation of the cell cycle.

A study at the Saint Louis University School of Medicine of USA has found that cholesterol suppresses the responsiveness of cardiovascular cells to TGF-β and its protective qualities, thus allowing atherosclerosis to develop. It was also found that statins, drugs that lower cholesterol levels, enhance the responsiveness of cardiovascular cells to the protective actions of TGF-ß, thus helping prevent the development of atherosclerosis and heart disease.

3-Hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) catalyzes the formation of mevalonate - converts HMG-CoA to mevalonic acid (Zhang et al., 2007). In many classes of organisms, this is the committed step leading to the synthesis of essential compounds, such as cholesterol. However, a high level of cholesterol is an important risk factor for

coronary heart disease, for which an effective clinical treatment is to block HMGCR using inhibitors like statins. Recently the structures of catalytic portion of human HMGCR complexed with six different statins have been determined by a delicate crystallography study (Zhang et al., 2007).

HMGCR inhibitors have been shown to upregulate GTP cyclohydrolase I (GTPCH-I), the key enzyme for tetrahydrobiopterin de novo synthesis and to normalize tetrahydrobiopterin levels in hyperglycemic endothelial cells (Wenzel et al., 2008).

The liver is responsible for controlling cholesterol homeostasis in the body. HMGCR and the LDL receptor (LDL-r) are involved in this regulation and are also ubiquitously expressed in all major tissues (Mutungi et al., 2007).

Statin reduces cerebrovascular events independent of its cholesterol lowering effect. Reduction of cerebrovascular events by statins may be brought by the direct inhibition of atherosclerotic change (Tsuchiya et al., 2007).

Since the description by Kimmelstiel and Wilson of the classical nodular glomerulosclerosis and presence of lipid deposits in the diabetic kidney (Kimmelstiel, 1936), several investigators have shown presence of lipid deposition in the kidneys of diabetic humans and experimental animals (Moorhead, 1982; Ruan, 2003; Sun, 2002; Jiang, 2005; Jiang, 2005; Bonnet, 2000; Spencer, 2004; Lee, 1991). The results from our longitudinal studies in ZDF rats indicate that at the initial stage of diabetic nephropathy, there are multiple disturbances in the lipid metabolic pathways and significantly increased lipid deposition in kidney, including cholesterol, triglyceride, ceramide and glucosylceramide. Analysis of transcriptional factors and their target enzymes that play an important role in regulation of lipid metabolism demonstrated significant a) augmentation of *de novo* fatty acid synthesis and b) concomitant decreased fatty acid oxidation in the initial stage of diabetic kidney development. Moreover, there was c) increased cholesterol synthesis and uptake, and d) decreased cholesterol efflux in the young ZDF rat kidney. Thus, the combined effects of these disturbances in renal lipid metabolism result in the net increased accumulation of lipids in the kidney. One of the novel and interesting findings in our present study is the demonstration of decreased FXR expression in ZDF rat kidney. FXR has been shown to inhibit SREBP-1c expression in the liver (Kalaany, 2006; Watanabe, 2004; Zhang, 2005). In the liver FXR has also been shown to induce fatty acid oxidation via stimulation of PPAR (Torra, 2003) and to have anti-fibrotic effect via decreasing TGF-M expression (Fiorucci, 2004; Fiorucci, 2005). Thus, the decrease in FXR activity in the kidney could mediate the upregulation of fatty acid synthesis, downregulation of fatty acid oxidation, and increased expression of TGF-M in the ZDF rat kidney.

In view of the toxic effects elicited by lipids on various target tissues and cells (Unger, 2003; Schaffer, 2003), we speculate that the ectopic accumulation of excess lipids in the kidney ultimately result in lipid-mediated cell injury or renal lipotoxicity. This could contribute to the pathogenesis of diabetic nephropathy. The lipotoxicity encompass various pathophysiological events including lipid-mediated cell injury. Lipotoxicity has been well documented in several non-adipose tissues including pancreatic cells (Shimabukuro, 1998), heart (Inger, 2005), liver (Shimomura , 1999) and skeletal muscle Cha , 2005), and has a profound impact in the pathogenesis and target organ damage in the metabolic syndrome (Unger, 2003; Schaffer, 2003; Shimabukuro, 1998; Cha, 2005; Shimomura, 1999; Unger, 2005).

It was observed that the increases in renal lipid content was already evident in 6 week old ZDF rats, prior to onset of hyperglycemia, glomerulosclerosis, and proteinuria indicating that these lipid alterations may play an important role in the progression of the diabetic renal injury. In support of this hypothesis, studies in renal mesangial and tubular cells grown in culture have shown that incubation of these cells with low density lipoprotein (LDL) or very low density lipoprotein (VLDL) cause upregulation of growth factors, including TGF-beta, PDGF (Nishida, 1997),. and plasminogen activator inhibitor-1 (PAI-1) (Song, 2005), extracellular matrix proteins, proinflammatory cytokines including interleukins and tumor necrosis factor-alpha (Nishida, 1997), adhesion molecules including monocyte chemotactic protein-1 (MCP-1), intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) (Di Paolo, 1999; Kamanna, 2002), and lipid peroxidation and glycoxidation (Lee, 1996), processes which play a role in the pathogenesis and progression of diabetic kidney injury. Our studies in ZDF rats indicate that *in vivo*, accumulation of lipids in the kidney is associated with a) increased expression of TGF-beta1, VEGF, PAI-1, b) increased expression of collagen and fibronectin, c) reduced expression of podocyte markers including podocin, ZO-1, and d) mesangial expansion. These functional and structural changes likely contribute to the development of glomerulosclerosis, tubulointerstitial fibrosis, and proteinuria. We provide evidence indicating that rosiglitazone decreases lipid accumulation in ZDF kidney by 1) prevention of fatty acid biosynthesis by suppression of nuclear SREBP-1 protein abundance; 2) induction of fatty acid oxidation via PPAR- alpha, PPAR-alpha, ACO and CPT-1 (Barish, 2006); 3) prevention of increased cholesterol

biosynthesis by suppression of nuclear SREBP-2 protein abundance (Goldstein, 2006); 4) prevention of LDL uptake via inhibition of elevated ox-LDLR expression (Lehrke, 2005; Chui, 2005); and 5) augmentation of cholesterol efflux via increased expression of ABCA1 (Akiyama, 2002; Ruan, 2003). The prevention of renal lipid accumulation was coupled with i) simultaneous decreases in the expression of profibrotic growth factors and proinflammatory cytokines including TGF-beta, VEGF, and IL-6, ii) prevention of extracellular matrix protein accumulation, and iii) prevention of podocyte injury and loss. These result in the significant amelioration of the development of glomerulosclerosis, tubulointerstitial fibrosis, and proteinuria.

Renal lipid metabolism may play important roles in renal inflammation, glomerulosclerosis and tubulointerstitial injury in diabetic nephropathy. These alterations in lipids were associated with (1) decreased expression of PPAR-γ mRNA and protein, (2) increased abundance of the sterol regulatory element binding protein-1 (SREBP-1), key regulator of fatty acid synthesis, (3) decreased abundance of farnesoid X receptor (FXR), a negative regulator of fatty acid synthesis and promoter of fatty acid oxidation, (4) downregulation of peroxisome proliferator-activated receptor delta (PPAR-γ), key regulator of fatty acid oxidation, (5) increased abundance of the sterol regulatory element binding protein-2 (SREBP-2), key regulator of cholesterol synthesis, and (6) downregulation of ATP binding cassette A1 (ABCA1), key regulator of cholesterol efflux. These lipid alterations were also associated with marked downregulation of the podocyte markers podocin and zonula occludens-1 (ZO-1) and proteinuria. Treatment of ZDF rats with the PPAR-γ agonist rosiglitazone resulted in normalization of the renal lipid metabolism pathways and prevention of lipid and adipophilin accumulation, restoration of podocin and ZO-1 expression, and prevention of proteinuria. Thus, our results indicate that renal lipid accumulation significantly contributes to renal cell injury and treatment with PPAR agonist significantly ameliorates podocyte injury, glomerulosclerosis, tubulointerstitial fibrosis, and proteinuria. Our data also suggests that the PPAR-FXR-SREBP pathway may play a critical role in regulation of lipid homeostasis and fibrosis in the kidney.

It was able to show that disturbance of renal expression of SREBP is involved in the development of nephropathy. Nuclear transcription factor SREBP-2 directly controls the gene transcription of 3-hydroxy 3-methylglutaryl coenzyme A reductase (HMG-CoA R), the later participates *de novo* cholesterol biosynthesis and serves as the working target for statins inhibition. Thus far, the mechanism of statin-mediated renal protection remains elusive, and the modulation of renal lipid metabolism pathway by atorvastatin (lipitor) hasn't been documented to our best knowledge.

PPAR-alpha agonists (thiazolidinedione or TZDs) have been shown to protect against the development of diabetic nephropathy in both human and animal models (Buckingham, 1998; Ma, 2001; Pistrosch, 2005). Nevertheless, the molecular mechanism underlying the TZD-mediated renal protection has not been fully characterized. Although effective normalization of hyperglycemia and hyperlipidemia by TZD treatment may play an important role in the prevention of renal complications of diabetes, several lines of evidence also support a direct role for TZDs on the kidney. For example, renal glomerular mesangial cells express PPAR-alpha receptors (Asano, 2000; Nicholas, 2001) and PPAR-alpha agonists have anti-fibrotic action in both *in vivo* and *in vitro* studies (Buckingham, 1998; Ma, 2001; Zafiriou, 2004; Zafiriou, 2005). In addition, PPAR-alpha agonists have been shown to be renal protective in models of type I diabetes, independent of any alterations in systemic blood glucose or lipid levels Isshiki, 2000). In the streptozotocin diabetic rat treatment with troglitazone was shown to prevent the increased expression of TGF-beta, fibronectin and type IV collagen. Troglitazone prevented the increase in glomerular diacylglycerol (DAG) content, protein kinase C (PKC) activity and ERK2 phosphorylation while inducing an increase in DAG kinase activity (Isshiki, 2000). Troglitazone and pioglitazone also had similar effects in cultured mesangial cells, as they both prevented the high glucose induced increases in DAG, PKC and ERK2 phosphorylation (Isshiki, 2000). In another study in the streptozotocin diabetic rat troglitazone prevented the increased expression of PAI-1 (Nicholas, 2001). Troglitazone has also been shown to be protective against glomerulosclerosis and proteinuria in the 5/6 nephrectomy model of nondiabetic renal disease, by preventing the increased expression of PAI-1 and TGF-beta (Ma, 2001). Altogether, these studies therefore indicate that in addition to their systemic effects, PPAR-alpha agonists also have direct renal effects and modulate diabetic and non-diabetic renal disease by multiple cellular mechanisms, including modulation of renal lipid metabolism as supported by our current study. Another intriguing finding of the current study is the demonstration of the significant lipid accumulation in the podocytes and the concomitant reduction of podocyte markers podocin and ZO-1 in the ZDF rat kidney, and the corrective effect of rosiglitazone. Podocyte injury is closely related to development and progression of diabetic nephropathy in humans

(Mundel, 2002; Ly, 2004; Kriz; Wolf, 2005; Nakamura, 2001).

Augmentation of podocyte proteins including podocin, nephrin, and ZO-1 by rosiglitazone demonstrates an important mechanism for the PPAR-alpha mediated decrease in proteinuria and renal protective effect in the setting of diabetes mellitus. In summary, we conclude that ZDF rats exhibit a primary alteration in renal lipid metabolism. The accumulation of triglyceride and cholesterol in the kidney glomerular and tubular cells is mediated via simultaneous increase in fatty acid synthesis and decrease in fatty acid oxidation, increase in cholesterol synthesis and uptake, and decrease in cholesterol efflux. The increase in lipid deposition is also associated with podocyte injury and increased expression of TGF-M, VEGF, PAI-1, IL-6, accumulation of extracellular matrix proteins, and proteinuria, suggesting the existence of renal lipotoxicity. Treatment of ZDF rats with the PPAR-alpha agonist rosiglitazone depletes the ectopic deposition of excess lipids in the kidney, and significantly ameliorates lipotoxicity-associated renal pathological abnormalities.

Discussion

Renal lipid metabolism may play important roles in renal inflammation, glomerulosclerosis and tubulointerstitial injury in diabetic nephropathy. Since the description by Kimmelstiel and Wilson of the classical nodular glomerulosclerosis and presence of lipid deposits in the diabetic kidney, several investigators have shown presence of lipid deposition in the kidneys of diabetic humans and experimental animals. Podocyte injury maybe closely related to development and progression of diabetic nephropathy in humans. Lipitor has significantly effects on the diabetic rats (Ma, et al, 2009).

Appendix: Information of Lipitor by Pfizer (http://www.lipitor.com)

LIPITOR- atorvastatin calcium trihydrate tablet, film coated, Parke-Davis Div of Pfizer Inc.

LIPITOR® (atorvastatin calcium) Tablets for oral administration, Initial U.S. Approval: 1996.

LIPITOR is an inhibitor of HMG-CoA reductase (statin) indicated as an adjunct therapy to diet to:

- Reduce the risk of MI, stroke, revascularization procedures, and angina in patients without CHD, but with multiple risk factors.
- Reduce the risk of MI and stroke in patients with type 2 diabetes without CHD, but with multiple risk factors.
- Reduce the risk of non-fatal MI, fatal and non-fatal stroke, revascularization procedures, hospitalization for CHF, and angina in patients with CHD.
- Reduce elevated total-C, LDL-C, apo B, and TG levels and increase HDL-C in adult patients with primary hyperlipidemia (heterozygous familial and nonfamilial) and mixed dyslipidemia.
- Reduce elevated TG in patients with hypertriglyceridemia and primary dysbetalipoproteinemia.
- Reduce total-C and LDL-C in patients with homozygous familial hypercholesterolemia (HoFH).
- Reduce elevated total-C, LDL-C, and apo B levels in boys and postmenarchal girls, 10 to 17 years of age, with heterozygous familial hypercholesterolemia after failing an adequate trial of diet therapy.

DOSAGE AND ADMINISTRATION

Dose range: 10 to 80 mg once daily.

Recommended start dose: 10 or 20 mg once daily.

Patients requiring large LDL-C reduction (>45%) may start at 40 mg once daily.

Pediatric starting dose: 10 mg once daily; maximum recommended dose: 20 mg once daily.

DOSAGE FORMS AND STRENGTHS

10, 20, 40, and 80 mg tablets.

CONTRAINDICATIONS

Active liver disease, which may include unexplained persistent elevations in hepatic transaminase levels.

Women who are pregnant or may become pregnant.

WARNINGS AND PRECAUTIONS

Skeletal muscle effects (e.g., myopathy and rhabdomyolysis): Risks increase when higher doses are used concomitantly with cyclosporine and strong CYP3A4 inhibitors (e.g., clarithromycin, itraconazole, HIV protease inhibitors). Predisposing factors include advanced age (> 65), uncontrolled hypothyroidism, and renal impairment. Rare cases of rhabdomyolysis with acute renal failure secondary to myoglobinuria have been reported. Advise patients to promptly report to their physician unexplained and/or persistent muscle pain, tenderness, or weakness. LIPITOR therapy should be discontinued if myopathy is diagnosed or suspected.

Liver enzyme abnormalities: Persistent elevations in hepatic transaminases can occur. Check liver enzyme tests before initiating therapy and as clinically indicated thereafter.

A higher incidence of hemorrhagic stroke was seen in patients without CHD but with stroke or TIA within the previous 6 months in the LIPITOR 80 mg group vs. placebo.

ADVERSE REACTIONS

The most commonly reported adverse reactions (incidence ≥ 2%) in patients treated with LIPITOR in placebo-controlled trials regardless of causality were: nasopharyngitis, arthralgia, diarrhea, pain in extremity, and urinary tract infection.

Endocrine Function

Increases in HbA1c and fasting serum glucose levels have been reported with HMG-CoA reductase inhibitors, including LIPITOR.

Statins interfere with cholesterol synthesis and theoretically might blunt adrenal and/or gonadal steroid production. Clinical studies have shown that LIPITOR does not reduce basal plasma cortisol concentration or impair adrenal reserve. The effects of statins on male fertility have not been studied in adequate numbers of patients. The effects, if any, on the pituitary-gonadal axis in premenopausal women are unknown. Caution should be

exercised if a statin is administered concomitantly with drugs that may decrease the levels or activity of endogenous steroid hormones, such as ketoconazole, spironolactone, and cimetidine.

CNS Toxicity

Brain hemorrhage was seen in a female dog treated for 3 months at 120 mg/kg/day. Brain hemorrhage and optic nerve vacuolation were seen in another female dog that was sacrificed in moribund condition after 11 weeks of escalating doses up to 280 mg/kg/day. The 120 mg/kg dose resulted in a systemic exposure approximately 16 times the human plasma area-under-the-curve (AUC, 0–24 hours) based on the maximum human dose of 80 mg/day. A single tonic convulsion was seen in each of 2 male dogs (one treated at 10 mg/kg/day and one at 120 mg/kg/day) in a 2-year study. No CNS lesions have been observed in mice after chronic treatment for up to 2 years at doses up to 400 mg/kg/day or in rats at doses up to 100 mg/kg/day. These doses were 6 to 11 times (mouse) and 8 to 16 times (rat) the human AUC (0–24) based on the maximum recommended human dose of 80 mg/day.

CNS vascular lesions, characterized by perivascular hemorrhages, edema, and mononuclear cell infiltration of perivascular spaces, have been observed in dogs treated with other members of this class. A chemically similar drug in this class produced optic nerve degeneration (Wallerian degeneration of retinogeniculate fibers) in clinically normal dogs in a dose-dependent fashion at a dose that produced plasma drug levels about 30 times higher than the mean drug level in humans taking the highest recommended dose.

Use in Patients with Recent Stroke or TIA

In a post-hoc analysis of the Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) study where LIPITOR 80 mg vs. placebo was administered in 4,731 subjects without CHD who had a stroke or TIA within the preceding 6 months, a higher incidence of hemorrhagic stroke was seen in the LIPITOR 80 mg group compared to placebo (55, 2.3% atorvastatin vs. 33, 1.4% placebo; HR: 1.68, 95% CI: 1.09, 2.59; p=0.0168). The incidence of fatal hemorrhagic stroke was similar across treatment groups (17 vs. 18 for the atorvastatin and placebo groups, respectively). The incidence of nonfatal hemorrhagic stroke was significantly higher in the atorvastatin group (38, 1.6%) as compared to the placebo group (16, 0.7%). Some baseline characteristics, including hemorrhagic and lacunar stroke on study entry, were associated with a higher incidence of hemorrhagic stroke in the atorvastatin group.

Pediatric Patients (ages 10–17 years)

In a 26-week controlled study in boys and postmenarchal girls (n=140, 31% female; 92% Caucasians, 1.6% Blacks, 1.6% Asians, 4.8% other), the safety and tolerability profile of LIPITOR 10 to 20 mg daily was generally similar to that of placebo.

DRUG INTERACTIONS

The risk of myopathy during treatment with statins is increased with concurrent administration of fibric acid derivatives, lipid-modifying doses of niacin, cyclosporine, or strong CYP 3A4 inhibitors (e.g., clarithromycin, HIV protease inhibitors, and itraconazole).

Grapefruit Juice

Contains one or more components that inhibit CYP 3A4 and can increase plasma concentrations of atorvastatin, especially with excessive grapefruit juice consumption (>1.2 liters per day).

Cyclosporine

Atorvastatin and atorvastatin-metabolites are substrates of the OATP1B1 transporter. Inhibitors of the OATP1B1 (e.g., cyclosporine) can increase the bioavailability of atorvastatin. Atorvastatin AUC was significantly increased with concomitant administration of LIPITOR 10 mg and cyclosporine 5.2 mg/kg/day compared to that of LIPITOR alone.

Gemfibrozil

Due to an increased risk of myopathy/rhabdomyolysis when HMG-CoA reductase inhibitors are co-administered with gemfibrozil, concomitant administration of LIPITOR with gemfibrozil should be avoided.

Other Fibrates

Because it is known that the risk of myopathy during treatment with HMG-CoA reductase inhibitors is increased with concurrent administration of other fibrates, LIPITOR should be administered with caution when used concomitantly with other fibrates.

Niacin

The risk of skeletal muscle effects may be enhanced when LIPITOR is used in combination with niacin; a reduction in LIPITOR dosage should be considered in this setting.

Rifampin or other Inducers of Cytochrome P450 3A4

Concomitant administration of LIPITOR with inducers of cytochrome P450 3A4 (e.g., efavirenz, rifampin) can lead to variable reductions in plasma concentrations of atorvastatin. Due to the dual interaction mechanism of rifampin, simultaneous co-administration of LIPITOR with rifampin is recommended, as delayed administration of LIPITOR after administration of rifampin has been associated with a significant reduction in atorvastatin plasma concentrations.

Digoxin

When multiple doses of LIPITOR and digoxin were co-administered, steady state plasma digoxin concentrations increased by approximately 20%. Patients taking digoxin should be monitored appropriately.

Oral Contraceptives

Co-administration of LIPITOR and an oral contraceptive increased AUC values for norethindrone and ethinyl estradiol

Warfarin

LIPITOR had no clinically significant effect on prothrombin time when administered to patients receiving chronic warfarin treatment.

Colchicine

Cases of myopathy, including rhabdomyolysis, have been reported with atorvastatin co-administered with colchicine, and caution should be exercised when prescribing atorvastatin with colchicine.

USE IN SPECIFIC POPULATIONS

Pregnancy

Pregnancy Category X.

LIPITOR is contraindicated in women who are or may become pregnant. Serum cholesterol and triglycerides increase during normal pregnancy. Lipid lowering drugs

offer no benefit during pregnancy because cholesterol and cholesterol derivatives are needed for normal fetal development. Atherosclerosis is a chronic process, and discontinuation of lipid-lowering drugs during pregnancy should have little impact on long-term outcomes of primary hypercholesterolemia therapy.

There are no adequate and well-controlled studies of atorvastatin use during pregnancy. There have been rare reports of congenital anomalies following intrauterine exposure to statins. In a review of about 100 prospectively followed pregnancies in women exposed to other statins, the incidences of congenital anomalies, spontaneous abortions, and fetal deaths/stillbirths did not exceed the rate expected in the general population. However, this study was only able to exclude a three-to-four-fold increased risk of congenital anomalies over background incidence. In 89% of these cases, drug treatment started before pregnancy and stopped during the first trimester when pregnancy was identified.

Atorvastatin crosses the rat placenta and reaches a level in fetal liver equivalent to that of maternal plasma. Atorvastatin was not teratogenic in rats at doses up to 300 mg/kg/day or in rabbits at doses up to 100 mg/kg/day. These doses resulted in multiples of about 30 times (rat) or 20 times (rabbit) the human exposure based on surface area (mg/m2).

In a study in rats given 20, 100, or 225 mg/kg/day, from gestation day 7 through to lactation day 21 (weaning), there was decreased pup survival at birth, neonate, weaning, and maturity in pups of mothers dosed with 225 mg/kg/day. Body weight was decreased on days 4 and 21 in pups of mothers dosed at 100 mg/kg/day; pup body weight was decreased at birth and at days 4, 21, and 91 at 225 mg/kg/day. Pup development was delayed (rotorod performance at 100 mg/kg/day and acoustic startle at 225 mg/kg/day; pinnae detachment and eye-opening at 225 mg/kg/day). These doses correspond to 6 times (100 mg/kg) and 22 times (225 mg/kg) the human AUC at 80 mg/day.

Statins may cause fetal harm when administered to a pregnant woman. LIPITOR should be administered to women of childbearing potential only when such patients are highly unlikely to conceive and have been informed of the potential hazards. If the woman becomes pregnant while taking LIPITOR, it should be discontinued immediately and the patient advised again as to the potential hazards to the fetus and the lack of known clinical benefit with continued use during pregnancy.

OVERDOSAGE

There is no specific treatment for LIPITOR overdosage. In the event of an overdose, the patient should be treated symptomatically, and supportive measures instituted as required. Due to extensive drug binding to plasma proteins, hemodialysis is not expected to significantly enhance LIPITOR clearance.

DESCRIPTION

LIPITOR is a synthetic lipid-lowering agent. Atorvastatin is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, an early and rate-limiting step in cholesterol biosynthesis.

Atorvastatin calcium is [R-(R*, R*)]-2-(4-fluorophenyl)-ß, δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate. The empirical formula of atorvastatin calcium is (C33H34 FN2O5)2Ca•3H2O and its molecular weight is 1209.42. Its structural formula is:

Atorvastatin calcium is a white to off-white crystalline powder that is insoluble in aqueous solutions of pH 4 and below. Atorvastatin calcium is very slightly soluble in distilled water, pH 7.4 phosphate buffer, and acetonitrile; slightly soluble in ethanol; and freely soluble in methanol.

LIPITOR Tablets for oral administration contain 10, 20, 40, or 80 mg atorvastatin and the following inactive ingredients: calcium carbonate, USP; candelilla wax, FCC; croscarmellose sodium, NF; hydroxypropyl cellulose, NF; lactose monohydrate, NF; magnesium stearate, NF; microcrystalline cellulose, NF; Opadry White YS-1-7040 (hypromellose, polyethylene glycol, talc, titanium dioxide); polysorbate 80, NF; simethicone emulsion.

CLINICAL PHARMACOLOGY

Mechanism of Action

LIPITOR is a selective, competitive inhibitor of HMG-CoA reductase, the rate-limiting enzyme that converts 3-hydroxy-3-methylglutaryl-coenzyme A to mevalonate, a precursor of sterols, including cholesterol. Cholesterol and triglycerides circulate in the bloodstream as part of lipoprotein complexes. With ultracentrifugation, these complexes separate into HDL (high-density lipoprotein), IDL (intermediate-density lipoprotein), LDL (low-density lipoprotein), and VLDL (very-low-density lipoprotein) fractions. Triglycerides (TG) and cholesterol in the liver are incorporated into VLDL and released into the plasma for delivery to peripheral tissues. LDL is formed from VLDL and is catabolized primarily through the high-affinity LDL receptor. Clinical and pathologic studies show that elevated plasma levels of total cholesterol (total-C), LDL-cholesterol (LDL-C), and apolipoprotein B (apo B) promote human atherosclerosis and are risk factors for developing cardiovascular disease, while increased levels of HDL-C are associated with a decreased cardiovascular risk.

In animal models, LIPITOR lowers plasma cholesterol and lipoprotein levels by inhibiting HMG-CoA reductase and cholesterol synthesis in the liver and by increasing the number of hepatic LDL receptors on the cell surface to enhance uptake and catabolism of LDL; LIPITOR also reduces LDL production and the number of LDL particles. LIPITOR reduces LDL-C in some patients with homozygous familial hypercholesterolemia (FH), a population that rarely responds to other lipid-lowering medication(s).

A variety of clinical studies have demonstrated that elevated levels of total-C, LDL-C, and apo B (a membrane complex for LDL-C) promote human atherosclerosis. Similarly, decreased levels of HDL-C (and its transport complex, apo A) are associated with the development of atherosclerosis. Epidemiologic investigations have established that cardiovascular morbidity and mortality vary directly with the level of total-C and LDL-C, and inversely with the level of HDL-C.

LIPITOR reduces total-C, LDL-C, and apo B in patients with homozygous and heterozygous FH, nonfamilial forms of hypercholesterolemia, and mixed dyslipidemia. LIPITOR also reduces VLDL-C and TG and produces variable increases in HDL-C and apolipoprotein A-1. LIPITOR reduces total-C, LDL-C, VLDL-C, apo B,

TG, and non-HDL-C, and increases HDL-C in patients with isolated hypertriglyceridemia. LIPITOR reduces intermediate density lipoprotein cholesterol (IDL-C) in patients with dysbetalipoproteinemia.

Like LDL, cholesterol-enriched triglyceride-rich lipoproteins, including VLDL, intermediate density lipoprotein (IDL), and remnants, can also promote atherosclerosis. Elevated plasma triglycerides are frequently found in a triad with low HDL-C levels and small LDL particles, as well as in association with non-lipid metabolic risk factors for coronary heart disease. As such, total plasma TG has not consistently been shown to be an independent risk factor for CHD. Furthermore, the independent effect of raising HDL or lowering TG on the risk of coronary and cardiovascular morbidity and mortality has not been determined.

Pharmacodynamics

LIPITOR, as well as some of its metabolites, are pharmacologically active in humans. The liver is the primary site of action and the principal site of cholesterol synthesis and LDL clearance. Drug dosage, rather than systemic drug concentration, correlates better with LDL-C reduction. Individualization of drug dosage should be based on therapeutic response.

Pharmacokinetics

Absorption: LIPITOR is rapidly absorbed after oral administration; maximum plasma concentrations occur within 1 to 2 hours. Extent of absorption increases in proportion to LIPITOR dose. The absolute bioavailability of atorvastatin (parent drug) is approximately 14% and the systemic availability of HMG-CoA reductase inhibitory activity is approximately 30%. The low systemic availability is attributed to presystemic clearance in gastrointestinal mucosa and/or hepatic first-pass metabolism. Although food decreases the rate and extent of drug absorption by approximately 25% and 9%, respectively, as assessed by Cmax and AUC, LDL-C reduction is similar whether LIPITOR is given with or without food. Plasma LIPITOR 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.

Distribution: Mean volume of distribution of LIPITOR is approximately 381 liters. LIPITOR is ≥98% bound to plasma proteins. A blood/plasma ratio of approximately 0.25 indicates poor drug penetration into red blood cells. Based on observations in rats, LIPITOR is likely to be secreted in human milk.

Metabolism: LIPITOR is extensively metabolized to ortho- and parahydroxylated derivatives and various beta-oxidation products. *In vitro* inhibition of HMG-CoA reductase by ortho- and parahydroxylated metabolites is equivalent to that of LIPITOR. Approximately 70% of circulating inhibitory activity for HMG-CoA reductase is attributed to active metabolites. *In vitro* studies suggest the importance of LIPITOR metabolism by cytochrome P450 3A4, consistent with increased plasma concentrations of LIPITOR in humans following co-administration with erythromycin, a known inhibitor of this isozyme.

13 NONCLINICAL TOXICOLOGY

Carcinogenesis, Mutagenesis, Impairment of Fertility

In a 2-year carcinogenicity study in rats at dose levels of 10, 30, and 100 mg/kg/day, 2 rare tumors were found in muscle in high-dose females: in one, there was a rhabdomyosarcoma and, in another, there was a fibrosarcoma. This dose represents a plasma AUC (0–24) value of approximately 16 times the mean human plasma drug exposure after an 80 mg oral dose.

A 2-year carcinogenicity study in mice given 100, 200, or 400 mg/kg/day resulted in a significant increase in liver adenomas in high-dose males and liver carcinomas in high-dose females. These findings occurred at plasma AUC (0–24) values of approximately 6 times the mean human plasma drug exposure after an 80 mg oral dose.

In vitro, atorvastatin was not mutagenic or clastogenic in the following tests with and without metabolic activation: the Ames test with *Salmonella typhimurium* and *Escherichia coli,* the HGPRT forward mutation assay in Chinese hamster lung cells, and the chromosomal aberration assay in Chinese hamster lung cells. Atorvastatin was negative in the *in vivo* mouse micronucleus test.

Studies in rats performed at doses up to 175 mg/kg (15 times the human exposure) produced no changes in fertility. There was aplasia and aspermia in the epididymis of 2 of 10 rats treated with 100 mg/kg/day of atorvastatin for 3 months (16 times the human AUC at the 80 mg dose); testis weights were significantly lower at 30 and 100 mg/kg and epididymal weight was lower at 100 mg/kg. Male rats given 100 mg/kg/day for 11 weeks prior to mating had decreased sperm motility, spermatid head concentration, and increased abnormal sperm. Atorvastatin caused no adverse effects on semen parameters, or reproductive organ histopathology in dogs given doses of 10, 40, or 120 mg/kg for two years.

CLINICAL STUDIES

Prevention of Cardiovascular Disease

In the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT), the effect of LIPITOR on fatal and non-fatal coronary heart disease was assessed in 10,305 hypertensive patients 40–80 years of age (mean of 63 years), without a previous myocardial infarction and with TC levels ≤251 mg/dL (6.5 mmol/L). Additionally, all patients had at least 3 of the following cardiovascular risk factors: male gender (81.1%), age >55 years (84.5%), smoking (33.2%), diabetes (24.3%), history of CHD in a first-degree relative (26%), TC:HDL >6 (14.3%), peripheral vascular disease (5.1%), left ventricular hypertrophy (14.4%), prior cerebrovascular event (9.8%), specific ECG abnormality (14.3%), proteinuria/albuminuria (62.4%). In this double-blind, placebo-controlled study, patients were treated with anti-hypertensive therapy (Goal BP <140/90 mm Hg for non-diabetic patients; <130/80 mm Hg for diabetic patients) and allocated to either LIPITOR 10 mg daily (n=5168) or placebo (n=5137), using a covariate adaptive method which took into account the distribution of nine baseline characteristics of patients already enrolled and minimized the imbalance of those characteristics across the groups. Patients were followed for a median duration of 3.3 years.

The effect of 10 mg/day of LIPITOR on lipid levels was similar to that seen in previous clinical trials.

LIPITOR significantly reduced the rate of coronary events [either fatal coronary heart disease (46 events in the placebo group vs. 40 events in the LIPITOR group) or non-fatal MI (108 events in the placebo group vs. 60 events in

the LIPITOR group)] with a relative risk reduction of 36% [(based on incidences of 1.9% for LIPITOR vs. 3.0% for placebo), p=0.0005]. The risk reduction was consistent regardless of age, smoking status, obesity, or presence of renal dysfunction. The effect of LIPITOR was seen regardless of baseline LDL levels. Due to the small number of events, results for women were inconclusive.

LIPITOR also significantly decreased the relative risk for revascularization procedures by 42%. Although the reduction of fatal and non-fatal strokes did not reach a pre-defined significance level (p=0.01), a favorable trend was observed with a 26% relative risk reduction (incidences of 1.7% for LIPITOR and 2.3% for placebo). There was no significant difference between the treatment groups for death due to cardiovascular causes (p=0.51) or noncardiovascular causes (p=0.17).

In the Collaborative Atorvastatin Diabetes Study (CARDS), the effect of LIPITOR on cardiovascular disease (CVD) endpoints was assessed in 2838 subjects (94% white, 68% male), ages 40–75 with type 2 diabetes based on WHO criteria, without prior history of cardiovascular disease and with LDL ≤ 160 mg/dL and TG ≤ 600 mg/dL. In addition to diabetes, subjects had 1 or more of the following risk factors: current smoking (23%), hypertension (80%), retinopathy (30%), or microalbuminuria (9%) or macroalbuminuria (3%). No subjects on hemodialysis were enrolled in the study. In this multicenter, placebo-controlled, double-blind clinical trial, subjects were randomly allocated to either LIPITOR 10 mg daily (1429) or placebo (1411) in a 1:1 ratio and were followed for a median duration of 3.9 years. The primary endpoint was the occurrence of any of the major cardiovascular events: myocardial infarction, acute CHD death, unstable angina, coronary revascularization, or stroke. The primary analysis was the time to first occurrence of the primary endpoint.

Baseline characteristics of subjects were: mean age of 62 years, mean HbA1c 7.7%; median LDL-C 120 mg/dL; median TC 207 mg/dL; median TG 151 mg/dL; median HDL-C 52 mg/dL.

The effect of LIPITOR 10 mg/day on lipid levels was similar to that seen in previous clinical trials.

LIPITOR significantly reduced the rate of major cardiovascular events (primary endpoint events) (83 events in the LIPITOR group vs. 127 events in the placebo group) with a relative risk reduction of 37%, HR 0.63, 95% CI (0.48, 0.83) (p=0.001). An effect of LIPITOR was seen regardless of age, sex, or baseline lipid levels.

LIPITOR significantly reduced the risk of stroke by 48% (21 events in the LIPITOR group vs. 39 events in the placebo group), HR 0.52, 95% CI (0.31, 0.89) (p=0.016) and reduced the risk of MI by 42% (38 events in the LIPITOR group vs. 64 events in the placebo group), HR 0.58, 95.1% CI (0.39, 0.86) (p=0.007). There was no significant difference between the treatment groups for angina, revascularization procedures, and acute CHD death.

There were 61 deaths in the LIPITOR group vs. 82 deaths in the placebo group (HR 0.73, p=0.059).

In the Treating to New Targets Study (TNT), the effect of LIPITOR 80 mg/day vs. LIPITOR 10 mg/day on the reduction in cardiovascular events was assessed in 10,001 subjects (94% white, 81% male, 38% ≥65 years) with clinically evident coronary heart disease who had achieved a target LDL-C level <130 mg/dL after completing an 8-week, open-label, run-in period with LIPITOR 10 mg/day. Subjects were randomly assigned to either 10 mg/day or 80 mg/day of LIPITOR and followed for a median duration of 4.9 years. The primary endpoint was the time-to-first occurrence of any of the following major cardiovascular events (MCVE): death due to CHD, non-fatal myocardial infarction, resuscitated cardiac arrest, and fatal and non-fatal stroke. The mean LDL-C, TC, TG, non-HDL, and HDL cholesterol levels at 12 weeks were 73, 145, 128, 98, and 47 mg/dL during treatment with 80 mg of LIPITOR and 99, 177, 152, 129, and 48 mg/dL during treatment with 10 mg of LIPITOR.

Treatment with LIPITOR 80 mg/day significantly reduced the rate of MCVE (434 events in the 80 mg/day group vs. 548 events in the 10 mg/day group) with a relative risk reduction of 22%, HR 0.78, 95% CI (0.69, 0.89), p=0.0002. The overall risk reduction was consistent regardless of age (<65, ≥65) or gender.

Abbreviations:

ABCA1, ATP binding cassette A1
AGE, advanced glycation end product
COX-2, cyclooxygenase-2
FAS, fatty acid synthase
FXR, farnesoid X receptor
HIF, Hypoxia-inducible factor
HMG-CoA R (HMGCR), 3-hydroxy 3-methylglutaryl coenzyme A reductase
IL, Interleukin
PCR, polymerase chain reaction
PPAR-γ, peroxisome proliferator-activated receptor delta
RT-PCR, real-time polymerase chain reaction
SREBP-1, sterol regulatory element binding protein-1
SREBP-2, sterol regulatory element binding protein-2
STZ, streptozotocin
TG, triglyceride
TGF, transforming growth factor
TNF, Tumor necrosis factors
VEGF, vascular endothelial growth factor
ZDF, Zucker diabetic fatty
ZO-1, zonula occludens-1

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