

Ruboxistaurin – A Promising Drug for Diabetic Retinopathy

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ABSTRACT

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Diabetic retinopathy is a common complication leading to visual disability. Laser photocoagulation and surgical procedures remains the mainstay of treatment in diabetic retinopathy. Presently there is no approved medical therapy available to delay the development or cure diabetic retinopathy. Ruboxistaurin, a Protein Kinase C-beta (PKC beta) inhibitor, is being developed as an effective treatment to prevent visual loss associated with diabetic retinopathy. Ruboxistaurin is an acyclic derivative of staurosporine belonging to a new class of N- (azacycloalkyl) bisindolylmaleimides. By inhibiting PKC beta, Ruboxistaurin prevents endothelial dysfunction in the retinal vessels in experimental models and displays antiangiogenic property by preventing intraocular neovascularization. In clinical trials, Ruboxistaurin showed no significant effects on the progression of diabetic retinopathy, but delayed the onset of visual loss, improved retinal circulation time and reduced the risk of visual loss. It has also been shown to be effective in patients with severe macular edema as it reduces the retinal leakage. In trials, the drug was well tolerated. Ruboxistaurin is under evaluation by FDA and once approved, would emerge as a promising therapeutic approach for treating diabetic retinopathy by preventing visual loss.

Keywords: PKC beta, Protein kinase C, Ruboxistaurin mesylate, LY333531, Maleimides, Macular edema, Staurosporine, Diabetic retinopathy

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INTRODUCTION

Diabetes mellitus is the most common endocrine disorder and is rightly called the 'silent killer'. It can lead to complications affecting multiple systems of the body. It is one of the commonest causes of blindness and visual disability in adults. Diabetic retinopathy is a microvascular complication seen in diabetic individuals that can result in the development of vitreous hemorrhage, macular edema and retinal detachment, resulting in blindness. The mainstay of treatment for diabetic retinopathy is glycemic control, laser photocoagulation and surgical procedures like vitrectomy. Current treatment of diabetic retinopathy is mostly surgical and there is need for developing medical treatments that can either halt or delay the progress of diabetic retinopathy.

Diabetic retinopathy consists of two stages – nonproliferative stage and proliferative stage. The International Classification of Diabetic Retinopathy devised by the

American Academy of Ophthalmology in 2001 divides diabetic retinopathy into Nonproliferative (Mild, Moderate, Severe and Very severe) and Proliferative (non-high-risk and high-risk) retinopathy.¹ Currently medical therapy is attempted to target the nonproliferative stage of diabetic retinopathy and surgical therapy is usually restricted to the proliferative stage. Advances in knowledge about the pathophysiological mechanisms underlying the development of retinopathy are paving way for the development of newer agents.

Altered glucose metabolism in diabetes produces a lot of biochemical changes in the retinal endothelial cell. Persistent hyperglycemia causes the activation of Diacyl glycerol (DAG)- Protein Kinase C (PKC) pathway. There are about 12 isoforms of PKC in the human body and the $\alpha 2$ isoform is predominantly activated in the retina.² The activation of PKC causes endothelial dysfunction and leads to the activation of a host of signaling pathways that induce VEGF (Vascular endothelial growth factor) resulting in the

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proliferation of endothelial cells. So PKC and VEGF are considered to be possible therapeutic targets for preventing diabetic retinopathy. Since VEGF induced endothelial proliferation is further dependent on PKC,² the PKC beta inhibitor Ruboxistaurin could emerge as an effective new approach for treating diabetic retinopathy.

Chemistry

Ruboxistaurin (LY333531), a 14-membered macrocyclic compound, is chemically (S)-9-((Dimethylamino)methyl)-6,7,10,11-tetrahydro-9H,18H-5,21:12,17-dimethenodibenzo(e,k)pyrrolo (3,4-h) (1,4,13)oxadiazacyclohexadecine-18,20(19H)-dione.^{3,4} It is an acyclic derivative of staurosporine belonging to a new class of N-(azacycloalkyl) bisindolylmaleimides.⁵ It has an empirical formula $C_{28}H_{28}N_4O_3$ and a molecular-weight of 468.547 g/mol. The boiling point is 744.4°C. It is given orally in the form of Ruboxistaurin mesylate, which is water-soluble. Figure 1 shows the chemical structure of Ruboxistaurin. Seven salts of Ruboxistaurin have been synthesized and evaluated (hydrochloride, mesylate, sulfate, succinate, acetate, phosphate, and tart rate). Based on the physicochemical properties like solubility, bioavailability, stability and purity, hydrochloride and mesylate salts were considered for preclinical studies. Later pre-clinical studies showed that mesylate salt was five times more soluble in water than hydrochloride salt, which was evident from the higher bioavailability observed for mesylate salt in dogs. Subsequently Ruboxistaurin mesylate monohydrate was chosen for further clinical development.

MECHANISM OF ACTION

In diabetes, there is increased activity of protein kinase C (PKC) found in the cell membranes. An increased concentration of intracellular Diacyl glycerol (DAG), an intermediary product of metabolism, is seen in

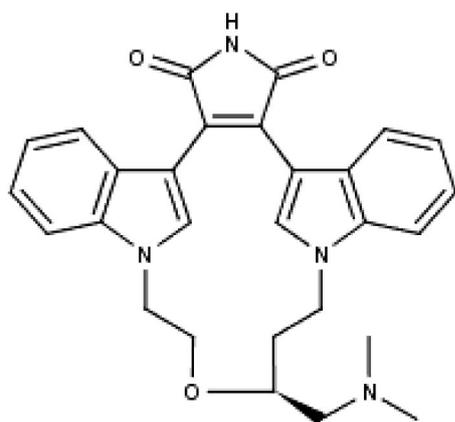


Figure 1. Chemical Structure of Ruboxistaurin

diabetes. Both isoforms of PKC beta have domains that can bind to DAG. This results in the activation of PKC beta, which can lead to the increased expression of cyclooxygenase, decreased production of the vasodilator prostacyclin and increased production of thromboxane A₂,⁶ decreased nitric oxide production and increased endothelin-1 (ET-1) production.⁷ PKC beta induces VEGF and causes proliferation and apoptosis of vascular smooth muscle cells also.⁸ All these contribute to endothelial dysfunction and diabetic retinopathy in diabetic patients. Increased leukocyte entrapment in the retinal vessels is also suggested to cause blood flow disturbances in the initial stages of diabetic retinopathy.

Ruboxistaurin is a competitive reversible inhibitor of PKC beta. Ruboxistaurin selectively inhibits both beta1 and beta2 isoforms of PKC beta⁵ and in comparison to PKC beta, there is 76 and 61 times inhibition of PKC beta1 and PKC beta2 respectively.⁴ Oral administration of Ruboxistaurin has been shown to prevent endothelial dysfunction in rats with streptozotocin-induced diabetes⁹ and in bovine retinal capillary endothelial cells.⁴ In diabetic rats, the onset of retinal hemodynamic changes like decreased blood flow is prevented by Ruboxistaurin.¹⁰ The endothelial dysfunction caused by hyperglycemia in human beings also is prevented by the inhibition of PKC beta.¹¹

Advanced glycation end products (AGE) play a key role in the development of vasculopathy in diabetes, manifested as endothelial cell dysfunction and inflammatory cells adhesion. Due to the involvement of PKC beta in the pathogenesis, it has been shown that PKC beta inhibition with Ruboxistaurin reduces AGE-induced macrophage adhesion to endothelial cells and secondarily, the local inflammation.¹²

Pharmacokinetic Parameters

Ruboxistaurin mesylate can be given orally. It is well absorbed from the GIT and well tolerated. Food enhances the absorption of Ruboxistaurin from the gastrointestinal tract.¹³ Ruboxistaurin is administered at 32 mg once daily. It is highly metabolized in the body and is excreted primarily through the bile and faeces (more than 80%), with urinary excretion constituting only a minor route.¹⁴ Ruboxistaurin is metabolized to the major metabolite, N-desmethyl Ruboxistaurin (metabolite 338522). This metabolite is equipotent to Ruboxistaurin in its inhibitory activity. CYP3A is the cytochrome enzyme primarily responsible for the production of this metabolite. Ruboxistaurin and its metabolites are found to inhibit CYP2D6.¹⁵ The half-life of Ruboxistaurin is nearly 9 hours and that

of the N- desmethyl metabolite is nearly 16 hours. Because of this long half-life, once daily administration is adequate. No dose adjustment is needed in patients with any degree of renal impairment since it is mainly excreted through the feces.¹⁴

Preclinical Studies

The efficacy of Ruboxistaurin in ameliorating the vascular dysfunction in diabetic retinopathy has been shown in many preclinical studies using *in vitro* and *in vivo* methods. Ruboxistaurin (LY333531) is shown to improve the retinal circulation in diabetic rats in a dose-responsive manner.¹⁰ In diabetic rat models induced by streptozotocin, Ruboxistaurin showed more than 50% reduction in the vascular complications produced by diabetes.⁹ In streptozotocin-induced diabetic mice models also, the drug was shown to reduce the nitric oxide- dependent vascular dysfunction as evidenced by an improvement in the nitric oxide mediated relaxation of aortic tissue and corpus cavernosum.¹⁶

The antiangiogenic property of ruboxistaurin was studied in pig models of preretinal neovascularization.¹⁷ Prevention of intraocular neovascularization was demonstrated by both fluorescein angiography and histopathological methods. In streptozotocin-induced diabetic rats, ruboxistaurin significantly reduced the number of leukocytes entrapped in the retinal microcirculation.¹⁸ Both these effects may contribute to the improvement of abnormal blood flow in the retinal vasculature seen associated with diabetes. In neonatal mice models for oxygen-induced retinopathy, ruboxistaurin inhibited retinal neovascularization via suppression of phosphorylation of ERK1/2 and Akt.¹⁹

Clinical Trials

Clinical trials have shown the efficacy and tolerability of ruboxistaurin in diabetic retinopathy patients. A double-blinded multicentric randomized placebo-controlled trial, named Protein Kinase C beta Inhibitor Diabetic Retinopathy Study (PKC- DRS) was conducted to evaluate the efficacy and safety of ruboxistaurin in subjects with moderately severe to very severe nonproliferative diabetic retinopathy.²⁰ 252 participants received either ruboxistaurin or placebo for 36– 46 months. Efficacy parameters were progression of retinopathy as assessed by the Early Treatment Diabetic Retinopathy Study (ETDRS) retinopathy severity level, moderate visual loss (MVL) as assessed by doubling of the visual angle and sustained MVL. Even though ruboxistaurin had no significant effects on the progression of diabetic retinopathy, it signifi-

cantly delayed the onset of moderate visual loss and significantly reduced the sustained MVL in those who had definite macular edema at the time of initiation of the study. This study concludes that even if ruboxistaurin does not prevent the progression of diabetic retinopathy, it reduces the risk of visual loss.

Another double-blinded randomized placebo controlled parallel group study showed that ruboxistaurin is well tolerated and it improves the retinal circulation time, thereby ameliorating the hemodynamic abnormalities seen in diabetic retinopathy.²¹ Some studies show that the drug is mostly effective in patients with severe macular edema as it reduces the retinal leakage in such patients.²²

A three-year long study, Protein Kinase C Diabetic Retinopathy Study (PKC-DRS2) trial, patients treated with placebo showed a decreasing visual acuity with increasing retinal thickness at the centre and increasing duration of centre-involving diabetic macular oedema.²³ The PKC-DRS2 study had a two-year open-label extension phase, where the investigators studied the effects of 32 mg/ day of ruboxistaurin in patients with moderate to severe nonproliferative diabetic retinopathy. RBX reduced the occurrence of sustained moderate visual loss to 5.5% compared to 9.1% in the placebo group.

Two clinical trials are going on in the United States to evaluate the efficacy of ruboxistaurin in reducing the occurrence of center-threatening diabetic macular edema (ClinicalTrials.gov Identifier: NCT00090519) and in reducing the macular thickness in patients with clinically significant macular edema (ClinicalTrials.gov Identifier: NCT00133952).

Indication

The drug is meant to be used for treating moderate to severe non-proliferative diabetic retinopathy at a dose of 32 mg once daily orally (proposed brand name is Arxxant).

Current Status

Phase III trials are going on with ruboxistaurin and FDA has sent an approvable letter in August 2006 to the innovator of this drug asking for more supporting data to support the clinical evidence presented in the New Drug Application (NDA). According to Eli Lilly, the innovator, FDA requested another 3-year Phase III clinical trial to get additional data on the molecule, which could delay the final approval of this molecule for another five years.²³

Adverse Effects

Even though no serious adverse effects have been attributed to ruboxistaurin, many adverse effects were reported from the clinical trials. These include abdominal pain, diarrhea, flatulence, asthma, proteinuria, nephropathy, dysuria, hyperkeratosis and first degree AV block.²⁰ There was no evidence of prolongation of cardiac conduction time. None of these adverse effects were significantly associated with ruboxistaurin therapy and it is well tolerated according to the data obtained from 937 patients included in two clinical trials, where the drug was given for 30 – 52 months.

Drug Interactions

The plasma concentrations of ruboxistaurin and N-desmethyl ruboxistaurin are reduced by CYP3A4 inducing agents like rifampicin, phenobarbitone and carbamazepine.¹³ Being a drug metabolized by CYP3A4, caution should be exercised when ruboxistaurin is co-administered with other CYP3A4 inhibitors like ketoconazole, erythromycin, verapamil, protease inhibitors and grape juice. Ruboxistaurin and its N-desmethyl metabolite are found to inhibit CYP2D6 in vitro and there is chance for drug interaction when drugs metabolized by CYP2D6 like tricyclic antidepressants, neuroleptics, selective serotonin reuptake inhibitors and opioids are administered to patients receiving ruboxistaurin.

Advantages

Once approved, it will be the first drug to be indicated for the treatment of diabetic retinopathy, which is a very common complication associated with diabetes.

It has shown some promising beneficial role in the treatment of diabetic nephropathy and diabetic neuropathy as well, in clinical trials.

Future Trends

Many other PKC beta inhibitors are also being studied in diabetic retinopathy. GF109203X, LY379196 and Ro31-8220 are a few of them. VEGF inhibitors like SU5416 (given systemically) and pegaptanib and rhuFab V2 (both given intravitreally) have also shown good results in diabetic retinopathy. Preliminary data from these studies show that these could become the possible drugs in future for treating diabetic retinopathy.

Additionally, Ruboxistaurin is being developed for diabetic neuropathy and diabetic nephropathy. With the experimental evidence showing benefits, the drugs may

also be evaluated in proliferative Glomerulonephritis and cardiac microvascular ischemia reperfusion injury.

CONCLUSION

With diabetes mellitus becoming a global menace and retinopathy being a very common complication associated with it, Ruboxistaurin can be a promising drug to improve the quality of life in these patients. As of the present situation, surgical procedures are adopted for treating diabetic retinopathy once the retinopathy has moved into the advanced stages involving macular edema and proliferative retinopathy. With the introduction of Ruboxistaurin in the market for treating non- proliferative diabetic retinopathy, there could be a medical treatment for the early stages of retinopathy. But the fact that this oral PKC beta inhibitor is not showing a role in preventing the progression of diabetic retinopathy shows that further research is needed for better agents that could prevent the progression of retinopathy in diabetics.

END NOTE

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Conflict of Interest: None declared

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