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PTP1B inhibitor Ertiprotafib is also a potent inhibitor of IκB kinase β (IKK-β)

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Abstract—Ertiprotafib was developed as an inhibitor of PTP1B for the treatment of type 2 diabetes. It normalized the plasma glucose and insulin levels in diabetic animal models, and progressed to a phase II clinical trial. Multiple in vivo targets of Ertiprotafib, in addition to PTP1B inhibition, have been suggested. In this study, Ertiprotafib was also shown to be a potent inhibitor of IκB kinase β (IKK-β), with an IC_{50} of 400 nM.

The worldwide prevalence of type 2 diabetes is increasing at an alarming rate.[1] Insulin resistance, an important factor in the pathogenesis of type 2 diabetes (DM2), is also related to metabolic diseases.[2] Thiazolidinediones (TZD) are a representative class of insulin sensitizers, which have been used for the treatment of DM2, as well as other diseases featuring insulin resistance.[3] Anti-diabetic drugs currently on the market also include agents that act by increasing the release of insulin (sulfonylurea, meglitinides), reducing hepatic glucose output (biguanides), increasing the release of glucagon-like peptide-1 (dipeptidyl peptidase-4 inhibitors) or inhibiting the digestion of food.[4] Most currently prescribed drugs are not free from side effects. The first TZD class of drug on the market, troglitazone, was withdrawn due to the increased risk of hepatitis. Edema, weight gain, and increased risk of heart failure have also been reported as side effects of TZD class anti-diabetic drugs.[4] In an attempt to develop a therapeutically efficient drug, with fewer side effects, a diverse range of novel targets have been studied.[5]

Recent studies have shown that deletion of the protein tyrosine phosphatase 1B (PTP1B) gene in mice produced healthy mice, with increased insulin sensitivity and resistance to diet-induced weight gain.[6] Similar results were obtained after the introduction of PTP1B antisense oligonucleotide into mice.[7] Based on these observations, PTP1B has emerged as a novel target for the treatment of diabetes and obesity, with numerous compounds having been developed as PTP1B inhibitors.[8] Of those compounds, only Ertiprotafib progressed to clinical trials prior to the discontinuation of a phase II due to the insufficient efficacy, coupled with unwanted side effects.[9]

Even though Ertiprotafib was developed as a potent PTP1B inhibitor, the biological response of mice treated with Ertiprotafib was found, in part, to be inconsistent with those observed with PTP1B-deficient or antisense PTP1B-treated mice. On feeding of a high-fat-diet, the free fatty acid (FFA) level in the serum of PTP1B-deficient mice was not lower than that in wild-type mice.[6b] Ertiprotafib treatment, however, significantly reduced the plasma FFA level in a rat obesity model.[10] These observations raised the possibility that the in vivo action of Ertiprotafib might not be limited to PTP1B inhibition. A recent study suggested that the therapeutic action of Ertiprotafib might involve the activation of PPARα and PPARγ, in addition to PTP1B inhibition.[10]

Keywords: Ertiprotafib; Diabetes; Inflammation; PTP1B; IKK-β; IκB kinase.
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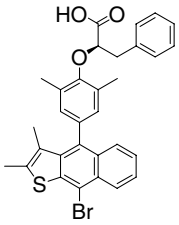

Figure 1. Chemical structure of Ertiprotafib.

With respect to the above suggestion, a recent study by Shoelson and colleagues on salicylate as an old therapy for diabetes drew our attention. They identified IκB Kinase β (IKK-β, encoded by *Ikbkb*) as a target of salicylate in relation to insulin sensitization.[11] Heterozygous *Ikbkb*$^{+/-}$ rodents exhibited a reduced level of fasting glucose and a protective effect against the development of insulin resistance.[11] Interestingly, the partial deletion of the *Ikbkb* gene in obese mice significantly reduced the level of plasma FFA, which was consistent with the result obtained in Ertiprotafib-treated mice.[11] These observations prompted us to examine the possibility that Ertiprotafib might exhibit its in vivo effect due to inhibition of the kinase activity of IKK-β Figure 1.

To measure the kinase activity of IKK-β, a peptide substrate, containing the phosphorylation motif of IκB, was used, with the phosphorylated peptide product determined using an ELISA-type assay employing time-resolved fluorescence (TRF) measurement.[12] With this technique, an europium ion was substituted for the amplifying enzyme coupled to the secondary antibody in the ELISA. A Eu^{3+} ion, complexed with a suitable ligand, was excited by a pulse of Laser, with the fluorescence emission measured after a short delay. Use of the TRF technique eliminates the background light level; thus, improving the sensitivity compared to classical fluorescence measurements.

As shown in Figure 2, Ertiprotafib was a potent inhibitor of IKK-β, with an IC_{50} value of 400 ± 40 nM, which was much lower than that required for the half-maximal inhibition of the *p*-nitrophenyl phosphatase activity of PTP1B. The reported IC_{50} value of Ertiprotafib against PTP1B ranged from 1.6 to 29 μM depending on the assay conditions;[10] an IC_{50} = 1.4 μM was obtained in our laboratory (unpublished result).

We also examined the inhibition of IKK-β by known inhibitors, Aspirin and sodium salicylate. In our assay conditions, significant inhibition of the enzyme activity was not observed up to 1 mM concentrations of both of the compounds (data not shown). Previously, Yin et al. observed an IC_{50} value of 30 ~ 50 μM with Aspirin and comparable inhibition with salicylate.[13] In numerous other studies, however, millimolar concentrations of Aspirin and salicylate were used to observe the in vivo effect of the drugs;[14] being consistent with our observations.

The IKK-β inhibition by Ertiprotafib added another possible mechanism to the complex nature of the

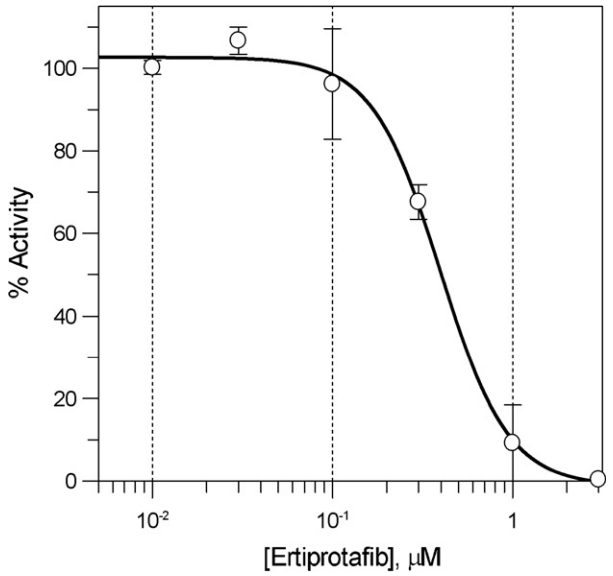

Figure 2. Dose-dependent inhibition of the kinase activity of IKKβ due to Ertiprotafib. The data points are the averages of duplicate reactions, and the error bars indicate the standard deviation. The data points were fitted using GraFit 5.0 (Erithacus Software Ltd., Horley, Surrey, UK) to calculate the IC_{50} value.

in vivo effect of Ertiprotafib.[10] IKK-β is well known as a target for the treatment of inflammatory diseases such as arthritis.[15] IKK-β is required for the activation of nuclear factor κB (NF-κB), which protects cells by suppressing apoptosis, but simultaneously results in local and systemic inflammation.[16] Therefore, the inhibition of IKK-β could be a therapeutic strategy for the treatment the inflammatory diseases. The improvement in insulin resistance due to the IKK-β inhibitor, Ertiprotafib, a potential anti-inflammatory drug, is consistent with the recent discovery that processes of insulin resistance and inflammation are linked.[17] In another study, obesity and high-fat-diet were shown to lead to hepatic inflammation through the activation of NF-κB and cause hepatic and systemic insulin resistance.[18] This result indicates that insulin resistance could be reversed by inhibition of the IKK-β/NF-κB pathway. Even though further study will be required to prove that the mechanism of action of Ertiprotafib involves the inhibition of IKK-β, this notion is at least consistent with recent study results.

In summary, Ertiprotafib was found to be a potent inhibitor of IKK-β, raising the possibility that in vivo effect of Ertiprotafib could be, in part, due to the inhibition of IKK-β.

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