Inhibition of mTOR Does Not Improve In Vivo Insulin Action in Ob/Ob or High Fat Fed Mice

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Abstract

The mammalian target of Rapamycin (mTOR) is a nutrient sensitive and insulin responsive Ser/Thr kinase that controls cell growth. It is not known if rapamycin, a highly specific inhibitor of mTOR, can improve glucose tolerance or insulin tolerance in genetically obese mice or in normal mice following five months of a HFD. Identical experiments were conducted in normal mice following five months of HFD or a standard chow diet (CON). Glucose tolerance was similar in mice fed the HFD and standard diet. Muscles were rapidly removed from mice 15 minutes following insulin injection and frozen in liquid nitrogen.

Introduction

• Type 2 diabetes is a metabolic disease associated with obesity and hyperinsulinemia (i.e., insulin resistance).
• Skeletal muscle insulin resistance is thought to be a primary defect in type 2 diabetes.
• The mammalian target of Rapamycin (mTOR) is a nutrient-sensitive and insulin responsive Ser/Thr kinase that controls cell growth.
• Cell culture studies demonstrate that mTOR promotes insulin resistance by the Ser phosphorylation and degradation of IRS-1.
• It is not known if rapamycin, a highly specific inhibitor of mTOR, can improve in vivo insulin action.

Purpose

The purpose of the present study was to determine the role of mTOR in insulin resistance and type 2 diabetes. We accomplished this by using Ob/Ob genetically obese (OB) mice and mice fed a high fat diet (HFD).

Methods

Animals

• Ob/Ob (n = 8) vs. Wildtype (WT, n = 8)
• 57/58 male mice fed HFD (n = 10) vs. standard chow diet (n=14)

Intraperitoneal glucose and insulin tolerance testing

• Rapamycin dose: 5.0 mg/Kg
• Glucose dose for GTT: 1.0 g/Kg
• Insulin dose for ITT: 0.25 U/Kg
• Blood was collected from the tail vein and glucose was assayed by glucometer (One Touch Ultra)

Figure 1. mTOR Signaling Pathway

Figure 2. GTT and ITT testing protocol

Muscle Processing and Immunoblotting

• 15 minutes following IP injection of insulin (25 U/Kg) or saline quadriceps muscles were removed rapidly frozen in liquid nitrogen.
• Frozen muscles were powdered and homogenized in cell extraction buffer (Biosource).
• Skeletal muscle extracts were prepared and subjected to SDS-polyacrylamide gel electrophoresis and immunoblotting reagents.
• Immunoblots were probed with the pThr389 S6K antibody, a phospho-specific antibody that recognizes only phosphorylated Thr389 of S6 Kinase (S6K). The Thr39 is the mTOR phosphorylation site.

Figure 3. The effect of rapamycin on phosphorylation of S6K kinase in skeletal muscle following insulin injection

Results

• Genotype Main Effect: P = 0.4833
• Rapamycin Main Effect: P = 0.7016
• Glucose Main Effect: P < 0.0001
• Genotype X Insulin Interaction: P < 0.0001

Conclusion

• Acute inhibition of mTOR with rapamycin does not improve insulin action in OB mice or mice fed a HFD.
• In the basal state, mTOR activity in skeletal muscle of OB mice is greater than WT mice.
• In the insulin stimulated state, mTOR activity in skeletal muscle of OB mice is similar to WT mice.
• Total IRS-1 levels in skeletal muscle are significantly lower in OB mice compared to wildtype mice, indicating that overactive mTOR signaling may promote IRS-1 degradation.

References