Section A-Research paper

ISOLATION, PURIFICATION AND ASSESSMENT OF ACTIVE COMPOUND MOLECULES AS POTENTIAL DRUGS TO CURE INSULIN RESISTANCE

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ABSTRACT

The development of NIDDM is mostly due to insulin resistance. This disorder develops when cells in the body do not react normally to insulin, despite having normal blood glucose levels. Type 2 diabetes may be effectively treated with medications that increase insulin receptor (IR) phosphorylation. They aid in enhancing the first stages of insulin signaling. The stem bark of Cinnamomum zeylanicum Blume was used to extract a proanthocyanidin called Cinnamtannin B1, which was then utilized in a research to determine whether or not it might phosphorylate IR. This chemical is a member of the Lauraceae genus. The chemical makeup of the substance was deduced using spectroscopy. Cinnamontannin B1 (0.11 mM) increases insulin receptor-subunit phosphorylation on 3T3-L1 adipocytes. Inhibition of GLUT4 by cytochalasin B and phosphatidylinositol 3-kinase (PI3K) activity by vanadate. Insulin receptor phosphorylation was unaffected by vanadate. These findings suggest that Cinnamtannin B1 stimulates the PI3K pathway, leading to the phosphorylation of IR.

Keywords: Mitochondrial superoxide dismutase, Cinnamomum zeylanicum, phosphorylation, cinnamtannin B1, insulin receptor, adipocyte, diabetes mellitus.

1. INTRODUCTION

Insulin, a hormone secreted by the pancreas, allows glucose to enter muscle, fat, and liver cells, where it is converted into energy. When you eat, your body breaks down the food you consume into glucose, a type of sugar. When you're fasting, the liver can also produce glucose to meet your body's demands. Following a meal, the pancreas discharges insulin into the bloodstream, which functions to decrease the blood glucose concentration. The regulation of blood sugar levels is accomplished by insulin, which facilitates the reduction of blood sugar levels. It is called "insulin resistance" when the insulin-producing cells in your muscles, adipose tissue, or liver are unable to readily absorb glucose from your blood. Pancreatic insulin production rises to help glucose reach your cells. [1] As long as you have enough insulin in your pancreas to overcome your cells' inability to respond, your blood sugar levels will remain in the healthy

range. Your pancreas produces extra insulin to compensate. A rise in blood sugar levels is inevitable over time. Insulin resistance (IR) is the term used to describe the condition where cells are unable to reply effectively to the insulin hormone.

Insulin aids glucose's entry into cells, decreasing blood sugar levels. When carbohydrates enter the bloodstream, the pancreas secretes insulin. People with insulin resistance respond differently to insulin, both in terms of glucose transport and blood sugar levels. Sulfate depletion may be crucial to insulin resistance, albeit the mechanism by which this occurs is not well known. [3] Some medications and medical conditions, as well as being overweight or inactive, can increase insulin resistance. Insulin resistance is characteristic of the metabolic syndrome. Doctors seldom employ fasting insulin levels and glucose tolerance testing, despite their usefulness in diagnosing insulin resistance. Changes in diet, exercise, and other elements of everyday life have been linked to better insulin sensitivity and even reversal of insulin resistance.

When insulin binds to insulin receptors in cells, a series of signaling cascades known as the PI3K/Akt/mTOR signalling pathway is activated. Recent research suggests that the insulin response may be a threshold phenomena, with certain cell types reacting to insulin as if it were a bistable switch under physiological settings. Lipolysis of free fatty acids is only one mechanism by which insulin sensitivity throughout the route might be reduced. However, organisms frequently employ sensitivity tuning (or lowering) to adapt to the dynamic nature of their environment or their metabolic needs. [2] Since both the mother's and the fetus's brains increase their glucose consumption during pregnancy, the mother's muscles become less sensitive to insulin. Insulin sensitivity may be improved, according to the adjustable threshold theory of insulin resistance, by inducing placental growth factor, which blocks the interaction between insulin receptor substrate (IRS) and PI3K.

Insulin resistance has been linked to the antioxidant defence mechanism of superoxide dismutase (SOD) in cell mitochondria, for various reasons. Studies have shown that mitochondrial superoxide dismutase mimics, electron transport chain inhibitors, and uncouplers may rapidly reduce insulin resistance. [4]

2. MATERIALS AND METHODS

• Chemicals

Sigma-Aldrich and Gibco BRL were our go-to spots for all of the research-grade chemicals we needed for our cell culture experiments. Merck-Schuchardt provided the high-quality chemicals used in the analysis. We used Merck (Darmstadt, Germany) chromatography-grade silica gel and thin-layer chromatography-specific silica gel (60 F254) in our experimentation. To supplement this, we ordered Sephadex-LH 20 from Pharmacia in Uppsala, Sweden. Promega (Madison, WI) provided the molecular

weight markers used to quantify protein concentrations. Both the main antibody (goat anti-rabbit IgG, HRP conjugated) and the secondary antibody (phosphotyrosine control, EGF-stimulated A431 cell lysate) were purchased from Upstate Biotechnology (Lake Placid, NY). The Shimadzu UV-100PC was used to capture ultraviolet spectra in methanol. The FT-IR Perkin Elmer 1600 was used to acquire IR spectra from KBr discs. The VG (Micromass) 70-SE mass spectrometer was used to gather the FAB-MS results. One hundred MHz of 13C NMR, three hundred MHz of 1H NMR, and one hundred and fifty MHz of 1H-1H COSY NMR were all recorded on a Bruker Avance spectrometer.

• Plant-Based Products

C. zeylanicum Blume was collected in West Sumatra, Indonesia. The Herbarium of Universitas Andalas, Padang, Indonesia, has placed a specimen (MT-09) as a specimen of record.

• Substance Purification and Isolation

During an 18-hour period, 750 grams of C. zeylanicum Blume stem bark in a dried and powdered form were extracted using n-hexane and acetone with a Soxhlet extractor. The 50g of acetone extract was analyzed by vacuum liquid chromatography on silica gel 60 (230-400 mesh, Merck) and eluted with a mixture of 1:1 methanol and ethyl acetate after the extraction procedure. TLC was employed to analyse each fraction (Silica gel 60 F254, Merck). Column chromatography and elution with ethyl acetate, acetone, and methanol were used to separate the fractions, among other techniques. The combined fractions with the same Rf were mixed, and the peptide fraction 6-20 was separated into 5 fractions, namely 4, 10, 11, 19, and 20-29, through silica gel column chromatography and elution with ethyl acetate and ethyl acetate: methanol (8:2). An amorphous, off-white material was produced after washing with 4-10 acetone fractions. To synthesize Chemical 1, the isolated chemical was purified using Sephadex LH-20 and eluted with methanol (0.6403 g, 0.085 percent). Sigma-Aldrich, Gibco BRL, and Mallinckrodt supplied all research chemicals. Merck (Darmstadt, Germany) supplied chromatography-grade and thin layer chromatography silica gel 60 F254. Promega (Madison, WI) sold protein-size molecular weight indicators. This study used goat anti-rabbit IgG, HRP conjugated, and phosphotyrosine control, EGF-stimulated A431 cell lysate from Upstate Biotechnology (Lake Placid, NY). The Shimadzu UV-100PC recorded ultraviolet spectra in methanol, while the FT-IR Perkin Elmer 1600 ran KBr disc IR spectra. A VG (Micromass) 70-SE mass spectrometer captured FAB-MS data, while a Bruker Avance 300 MHz spectrometer recorded 13C (100 MHz), 1H NMR (300 MHz), and 1H-1H COSY NMR spectra.

• Acetylation of Compounds

Ac_2O/pyridine was used to acetylate 60 mg of the compound (1:1). It took 24 hours of vigorous stirring at room temperature for the mixture to come together. Ethyl acetate was used to extract the combination from ice-cold distilled water. The light brown solid acetate derivative (58 mg) was obtained by evaporation and Sephadex LH-20 column chromatography, with elution in a 1:1 hexane/ethyl acetate combination.

• Cell Cultures

To culture and maintain 3T3-L1 adipocyte cells, they were grown in DMEM supplemented with 10% fetal bovine serum, 1% penicillin, and 1% streptomycin at 37°C in a humidified 5% CO2 environment. From day four, cells were incubated with differentiation medium containing 0.25 mM dexamethasone, 0.5 mM 1-isobutyl-3-methylxanthine, and 1 mM insulin, with media changes every two days. After dexamethasone and IBMX removal, cells were maintained in insulin-containing media for two days. In DMEM with 10% fetal bovine serum, differentiation occurred on days 9-14 of the induction phase.

- **Assay for Enzyme-Linked Immunosorbent Particles**

96-well plates contained 2 x 103 3T3-L1 preadipocytes. 2 days after confluence, adipocyte differentiation was triggered. After 11 days of induction, cells received 100 nM insulin and 0.11 mM cinnamtannoin B1 for 30 minutes. 100 µL of 50:50 methanol and acetic acid fixed cells for 10 minutes. Inverting and tapping the plate eliminated extra PBS after three PBS rinses. Each well was blocked with 200 µL of blocking solution (3 percent skim milk in PBS) for 1 hour at 37°C to avoid non-specific antibody binding. Each well received antiphosphotyrosine antibody diluted in blocking buffer after pressing the plate to remove excess PBS. overnight at 4°C. After three washes with PBS-Tween 0.05%, each well received an HRP-conjugated secondary antibody in blocking buffer (1:5000) for 1 hour at room temperature. After three washings with washing solution, cells were treated with 2,2'-azino-bis(3-ethylbenzthiazoline6-sulfonate (ABTS) reagent (KPL, Gaithersburg, MD) for 30 minutes at room temperature. Each well received 50 µL of 4% sodium dodecyl sulfate (SDS) to terminate the reaction, and a Bio-Rad ELISA plate reader scanned the plate at 410 nm.

3. RESULTS AND ANALYSIS

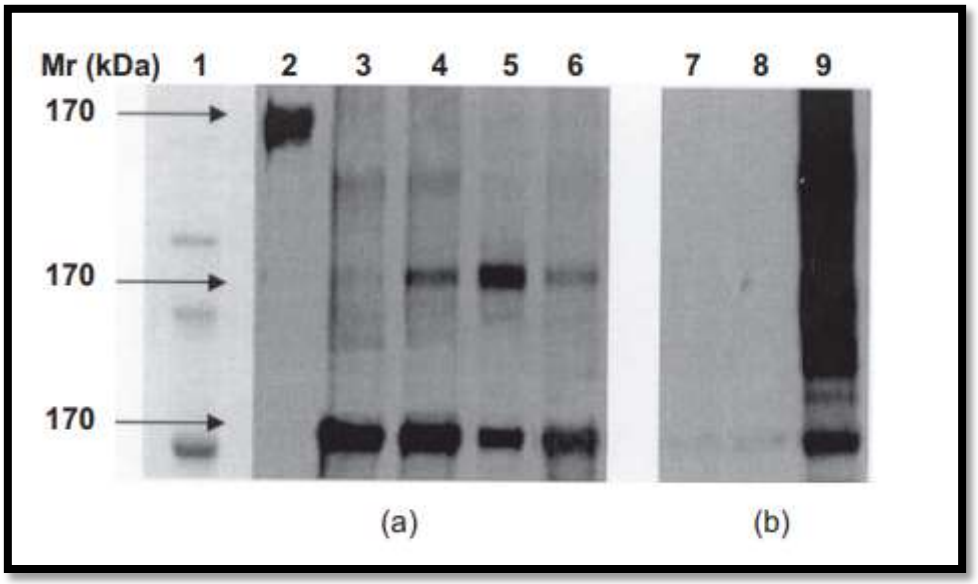

Fig 1. Cinnamtannin B1 has an effect on insulin receptor-subunit phosphorylation.

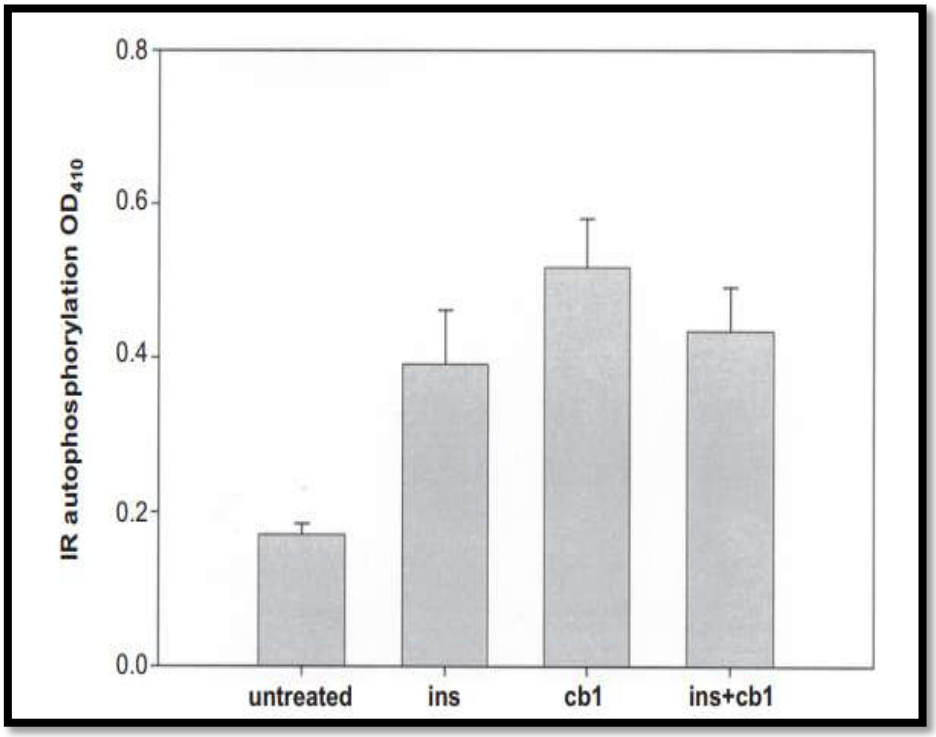

Fig.2 ELISA for insulin receptor phosphorylation in intact cells

4. DISCUSSIONS

Individuals with type 2 diabetes who are not insulin-dependent may have uncontrolled diabetes mellitus due to insulin resistance. Promising results have been seen in treating type 2 diabetes with drugs that promote autophosphorylation of insulin receptors (IR) and consequently activate IR signaling in the body. The glucose transport mechanism may require a series of events that start with receptor activation, such as insulin binding, receptor autophosphorylation, and endogenous substrate phosphorylation mediated by a receptor. The amount of tyrosine phosphorylation at the insulin receptor was dramatically enhanced by cinnamontannin B1. The binding of insulin activates tyrosine kinase, which results in the autophosphorylation of numerous Tyr residues found within the cell. Tyrosine residues on the IRS protein are also phosphorylated in this process.

5. CONCLUSION

I discovered cinnamontannin B1, a bioactive substance, in C. zeylanicum. It seems to have an insulin-like action by attaching to a receptor tyrosine residue in the cell membrane and causing the tyrosine residues to self-phosphorylate. Insulin is a hormone that regulates blood sugar levels. Autophosphorylation activates PI3K, GLUT4 translocation, and glucose absorption. Studies have demonstrated that cinnamtannin B1 is involved in inhibiting insulin receptor-subunit phosphorylation, which is an important target for diabetes treatment.

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