Original Article

Activating Effect of Momordin, Extract of Bitter Melon (*Momordica Charantia* L.), on the Promoter of Human PPAR δ

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Aim: Bitter melon (*Momordica charantia* L.) is a common vegetable grown in Okinawa that has also been used recently in medicine for the treatment of diseases such as diabetes, hypertension, and dyslipidemia. Among Bitter melon extracts compounds, we focused on an extract known as momordin in the present study, to examine its effect on peroxisome-proliferator activated-receptor (PPAR) δ (also called PPAR δ in rodents) expression and promoter activity of the human PPAR δ gene.

Methods: A human PPAR δ promoter-reporter plasmid was made as a template from a BAC CLONE (RPCI-11C) containing a -3076 bp (BglI site) +74 bp (EcoRI site) sequence. Luciferase assay of PPAR δ promoter activity was performed using HepG2 cells.

Results: 10 and 25 nM Momordin significantly increased the expression of PPAR δ mRNA 1.5-fold (relative to the control). Moreover, 10 and 25 nM Momordin significantly increased PPAR δ promoter activity in a dose-dependent manner, reaching more than 1.5-fold relative to the control.

Conclusion: Our present data obtained through successful cloning of the PPAR δ promoter demonstrate that PPAR δ production and activation are upregulated through PPAR δ promoter activity following momordin treatment.

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Key words; Bitter melon, PPAR β/δ , Momordin, PPAR β/δ promoter

Introduction

Peroxisome proliferator-activated receptor (PPAR) β/δ is the least well-defined subtype among the PPARs. Recent biological studies have disclosed that its activation significantly increases high-density lipoprotein (HDL)-cholesterol levels, and it influenced glycemic control in a primate model of metabolic syndrome¹⁻³. Recent studies also suggest that the overexpression of PPAR β/δ in adipose tissue protects against diet-induced obesity in mice, and treatment with a PPAR β/δ -selective agonist reduces weight gain without affecting food intake in fat-fed mice⁴. During some synthetic ligands of PPAR, have been reported little is known about the

PPAR β/δ agonist in extracts of natural foods.

Bitter melon, recognized as having medicinal value in India and traditional Chinese medicine, is a common vegetable in tropical areas. This vegetable showed a hypoglycemic effect in diabetic rats as well as in human subjects with type 2 diabetes mellitus but the mechanism for the hypoglycemic and hypolipidemic effects of bitter mellon is still unclear. It has numerous other constituents, including proteins momordin, glycosides, saponins, vitamin A, vitamin C, β -carotene and minerals⁵.

Momordin might preventing diabetes, hypertension and dyslipidemia, which involves activated c-Jun NH₂-terminal kinase (JNK). Momordin inhibits activator protein-1 (AP-1) activation induced by phorbol ester⁶. JNK signaling pathways are stimulated through AP-1 activation⁷. Activated JNK could be critical in causing diabetes, insulin resistance and obesity⁸. Moreover, momordin also showed inhibitory action against both jun/jun homodimer and jun/fos het-

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erodimer. Thus, we selected momordin as a nuclear factor modulator among many kinds of compounds in bitter melon.

In the present study, we investigated the effect of momordin on the regulation of PPAR β/δ expression in promoter activity of the human PPAR β/δ gene.

Materials and Methods

Reagents and Cell Culture

Momordin (Code number: 10-118) was purchased from Inland Laboratories (Import agent: Wako Pure Chemical Industries, Ltd., Tokyo, Japan). Momordin was dissolved in dimethyl sulfoxide (DMSO). In all assays, the final concentrations of DMSO were less than 0.5%. The human hepatoblastoma cell line (HepG2 cells) was purchased from JCRB (cell number: JCRB1054) cultured in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen) containing 10% heat-inactivated fetal bovine serum (FBS) (JRH Biosciences) and PNS Antibiotic Mixture (Invitrogen) at 37°C in 5% CO₂.

Cloning of the PPAR β/δ Promoter and Plasmid Constructions

To generate human PPAR β/δ promoter-reporter plasmid, we referred to the genomic sequence that has been reported previously⁹. Human PPAR promoter-reporter plasmid was made as template form BAC CLONE (RPCI-11C), containing -3076 bp (BglI site) +74 bp (EcoRI site). The transcriptional initiation site of PPAR was selected (Gene Bank accession NM_006238, NM_001039694). Moreover, a series of deletion constructs was produced using PCR or restriction sites in all promoters.

Luciferase Assay of PPAR β/δ Promoter Activity

HepG2 cells were transfected using LipofectamineTM 2000 (Invitrogen) according to the manufacturer's protocols. The cells (1×10^5 cells/well) were seeded in 24-well plates (Falcon) and incubated for 18 hours before transfection, using LipofectamineTM 2000 with 1 μ g human PPAR β/δ promoter-reporter plasmid and 0.1 μ g pRL-TK (Promega), a renilla luciferase reporter vector as an internal control for transfection efficiency. After 3 hours, the transfection medium was replaced by 10% FBS-DMEM plus various amounts of momordin (0, 0.1, 0.2, 0.5, 1, 2, 5, 10, 25 nM) or vehicle (DMSO or distilled water) and the cells were incubated for 24 hours. Luciferase activities were quantified using a Dual-Luciferase[®] Reporter Assay System (Promega) according to the manufacturer's protocols.

Real-Time Reverse Transcription (RT)-PCR Analysis

For PPAR β/δ , HepG2 cells (2×10^5 cells/dish) were incubated with various amounts of momordin (0, 0.1, 0.2, 0.5, 1, 2, 5, 10, 25 μ M) at 37°C for 24 hours. After treatment with momordin, cells were homogenized in 1 mL ISOGEN (Nippongene), and then total RNA was extracted with chloroform and precipitated with ethanol. First-strand cDNA was generated from total RNA with random hexamers and MuLV transcriptase (Applied Biosystems) according to the manufacturer's protocols. PCR reactions were performed with TaqMan[®] Universal PCR Master Mix and TaqMan[®] Gene Expression Assays (Applied Biosystems). Identification numbers of the assay mixture of target gene-specific primers and probes were as follows: PPAR, Hs00606407_m1; 18S ribosomal RNA (house-keeping gene), Hs99999901_s1. Real-time PCR reactions were performed with thermal cycling conditions of 2 minutes at 50°C, 10 minutes at 95°C, and 40 cycles of 15 seconds at 95°C and 1 minute at 60°C using the ABI PRISMTM 7900HT Sequence Detection System (Applied Biosystems). PPAR levels were normalized to 18S ribosomal RNA levels, and are presented as fold differences of momordin treated cells compared with untreated cells.

Statistical Analysis

All data are presented as the means $\pm$ SEM. Statistical analysis was performed using ANOVA followed by the Dunnett test or Scheffe test (StatView software). Statistical significance was considered as p value < 0.05 .

Results

Momordin Increased PPAR mRNA Expression

Firstly, we examined the effect of momordin on PPAR β/δ mRNA expression in HepG2 cells. We decided the appropriate incubation time and dose, as shown in **Fig. 1, 2**. The time-course study for PPAR β/δ mRNA expression in HepG2 cells treated with 10 nM momordin is shown in **Fig. 1**. Momordin significantly increased PPAR β/δ mRNA expression by 1.5-fold, 1.3-fold (versus the control) at 12 and 24 hours, respectively. We next examined the effect of various amounts of momordin for 24 hours on PPAR δ mRNA expression in HepG2. In **Fig. 2**, momordin (10 and 25 nM) significantly increased PPAR δ mRNA expression by 1.5-fold (versus the control).

On the other hand, higher concentrations of momordin treatment (50, 100 and 250 nM) did not increase PPAR β/δ mRNA expression significantly (data not shown).

Fig. 1. Effects of momordin incubation time on PPAR mRNA expression by RT-PCR analysis.

10 nM momordin significantly increased PPAR β/δ mRNA expression at 12 and 24 hours. All data are presented as the means $\pm$ SEM. * p < 0.05 vs 0 hour

Fig. 2. Effects of momordin on PPAR mRNA expression by RT-PCR analysis.

Momordin significantly increased PPAR β/δ mRNA expression dose-dependently at 24 hours. All data are presented as the means $\pm$ SEM. * p < 0.05 vs 0 nM

Fig. 3. Effects of momordin on human PPAR δ promoter activity.

Momordin significantly increased PPAR δ promoter activity in a dose-dependent manner. All data are presented as the means $\pm$ SEM. * p < 0.05 vs 0 nM

Momordin Increased Human PPAR δ Promoter Activity

To investigate the mechanism by which momordin increases PPAR δ mRNA expression, we cloned the human PPAR δ promoter region (−3076 to +74 bp) and examined promoter activity in HepG2 cells transfected with the human PPAR δ promoter-reporter plasmid. **Fig. 3** shows PPAR δ promoter activity following treatment of HepG2 cells with various amounts of momordin for 24 hours. Momordin significantly increased PPAR δ promoter activity in a dose-dependent manner by more than 1.5-fold (versus the control).

Discussion

The present study was undertaken to evaluate the effect of momordin (extract of *Momordica charan-*

tia) by our successful cloning of the PPAR β/δ promoter. In our results, momordin regulated PPAR δ production through PPAR δ promoter activity. Bitter melon (*Momordica charantia* L.), which has hypoglycemic and hypotriglyceridemic effects¹⁰⁾, is a common vegetable in Okinawa that has also been used recently in medicine for the treatment of diabetes, hypertension and dyslipidemia^{11, 12)}; however, the mechanism and active compounds responsible for these biological effects of bitter melon have not been fully elucidated. Bitter melon also has numerous other constituents, including proteins, momordin, glycosides, saponins, and minerals. It is also rich in vitamins A and C and β -carotene, as well as iron, phosphorus, and potassium⁵⁾.

In this study, we revealed that momordin has anti-glycemia and anti-lipidemia effects on target genes of PPAR β/δ -regulated PPAR β/δ promoter activity.

The effect of momordin on nuclear factors is interesting. It has also been reported that momordin suppressed osteoclastogenesis through the inhibition of NF- κ B and AP-1¹³⁾. In animal experiments, momordin showed both the inhibition of AP-1 transcriptional activity and cellular cytotoxicity. By accumulating data for nuclear factor regulation by momordin, further study may reveal the unknown mechanisms of momordin¹⁴⁾.

Telmisartan, an angiotensin II receptor antagonist with partial PPAR γ agonist activity, modulates the metabolism involving atherogenesis^{15, 16)}. Other drugs also have a mechanism involving the PPARs system¹⁷⁻²⁰⁾. The generation of monoclonal antibodies against human peroxisome proliferator-activated receptors²¹⁾ has developed new treatment by clarifying atherogenesis²²⁾. Although the action of each PPARs is interesting, PPAR δ modulator is not currently available as a drug. Many studies have revealed that PPAR δ controls the metabolic genes involved in glucose and lipid homeostasis. (i.e., mitochondrial β -oxidation/peroxisomal β -oxidation-related genes, UCP (uncoupling protein) 2, glucose oxidation, PDK (pyruvate dehydrogenase kinase) 4, mitochondrial HMG-CoA synthase etc.)²³⁻²⁶⁾; therefore, momordin may be used as a natural PPAR δ modulator.

Care should be taken regarding the concentration of this agent. A higher concentration (100 nM) of momordin, which act as a ribosome-inactivating protein (RIP)²⁷⁾, induced apoptosis in a bioassay²⁸⁾. In our results, a higher concentration of momordin did not increase PPAR δ expression and promoter activity, but it is uncertain whether the concentration of momordin used in our experiment is physiological.

More investigation may reveal the effect of PPARs on not only atherogenesis but also other diseases²⁹⁾.

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